GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

OCA

Date: 7/28/78

Project Title: The Anti-Tumor Agent of Aplopappus Heterophyllus

Project No: G-33-C03

Project Director: Dr. Leon H. Zalkow

Sponsor: DHEW/PHS/NIH - National Cancer Institute

Agreement Period: From 7/1/78 Until 6/30/79 (03 year)

Type Agreement: Grant No. 5 R01 CA18819-03

Amount: $52,909 PHS Funds (G-33-C03)
8,903 GIT Contribution (G-33-328)
$61,812 Total

Reports Required: Annual Progress Reports with Continuation Applications;
Terminal Progress Report upon Grant Expiration

Sponsor Contact Person (s):

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NOTE: FOLLOW-ON PROJECT TO G-33-C02
(02 Year)

Phone: (301) 496-7444

Defense Priority Rating: None

Assigned to: Chemistry (School/Laboratory)

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GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION SHEET

Date 5/5/83

Project Title: The Anti-Tumor Agent of Aplopappus Heterophyllus

Project No: G-33-C03

Project Director: Dr. Leon H. Zalkow

Sponsor: DHEW/PHS/NIH - National Cancer Institute

Effective Termination Date: 8/31/79 (03 year)

Clearance of Accounting Charges: 8/31/79

Grant/Contract Closeout Actions Remaining:

- ☐ Final Invoice and Closing Documents
- ☐ Final Fiscal Report
- ☐ Final Report of Inventions
- ☐ Govt. Property Inventory & Related Certificate
- ☐ Classified Material Certificate
- ☐ Other _____

It appears issuance of Final Technical Report 4/26/83 completes closing requirements on this grant. Invention statement issued 8/21/80.

Assigned to: Chemistry (School/~~Laboratory~~)

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FORM OCA 10:781

Proj. No.
G-33-C03

"THE ANTITUMOR AGENT OF APLOPAPPUS HETEROPHYLLUS"

CA-18819

TERMINAL PROGRESS REPORT

6/30/76-8/31/79

Leon H. Zalkow

School of Chemistry

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Atlanta, Georgia 30332

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255-36-9515

Summary: We have further partitioned and separated by chromatography the active chloroform fractions of I. Wrightii collected from two different sites in New Mexico and have identified six benzofuran derivatives and one coumarin. Three of these were previously unreported structures and none had been previously screened. Some show low level anti-tumor activity. The volatile oil of the plant shows anti-feedant activity against the fall armyworm and contains three new sesquiterpenes of previously unreported skeleta. The steroid fraction contains biogenetically important $\Delta^{8(14)}$ stigmasterols. The plant contains a small alkaloid fraction which has so far defied separation and a large number of apparently unknown compounds, nine of which have been isolated in pure form and partly characterized. Two new benzofurans have been isolated from Eupatorium rugosum in addition to a number of previously reported compounds. E. rugusum and I. Wrightii produce a clinically identical disease in higher animals. The absolute configuration of toxol(2S-isopropenyl-3R-hydroxy-5-a acetyl-2,3-dihydrobenzofuran) and its congeners has been firmly established by an X-ray analysis of a synthetic compound, nmr studies and chemical degradations.

Detailed Report:

I. The Benzofurans

The benzofurans dehydrotremetone (3), toxyl angelate (6), 2,5-diacetylbenzofuran (5), toxethol (7), and toxol (4) were isolated, in the order given by chromatography of the acid-free aq MeOH fraction on acid-washed Al_2O_3, Act III. This fraction was obtained as follows: The dried leaves and flowers of I. Wrightii were defatted with hexane, then extracted with 95% EtOH. The latter extract, after removal of solvent, was partitioned between $CHCl_3$ and H_2O and the $CHCl_3$ fraction, after removal of solvent, was further partitioned between aq MeOH (1:9) and hexane. The aq MeOH fraction, after removal of solvent, was dissolved in ether and this

solution was washed with cold aq 5% NaOH solution. While glc indicated, in addition to the above mentioned benzofurans (3-7), the presence of tremetone (1), it was isolated by a different procedure as follows. The ethanol extract of the entire above ground plant material, after initial defatting with hexane, was saponified using KOH (~ 10%) in aq MeOH (1:1) to yield an ether soluble "red jelly," analogous to the original rayless goldenrod "tremetol" of Couch (5).

This "red jelly" was separated into ketone and non-ketone fractions using Girard's T reagent. GLC of the ketone fraction showed three major components of increasing retention time in the ratios of 1.3 : 1 : 5.3 identified as dehydrotremetone (3), tremetone (1) and toxol (4), respectively, which were isolated by chromatography on Si gel. Toxyl angelate (6) was most readily isolated from the original hexane extract of the entire above ground plant material as follows. The hexane extract was steam distilled and the ether soluble non-volatile residue was distributed between benzene and aq EtOH. The benzene fraction after washing with cold 5% NaOH was chromatographed on Al_2O_3(act II) and then Si gel to give toxyl angelate (6).

The structure of toxyl angelate (6) was surmised from analysis of ^{1}H and ^{13}C NMR spectra and its mass spectrum and confirmed by its hydrolysis to the known toxol (4). Likewise the structure of toxethol (7), while being partially suggested from its spectral properties, was firmly established, except for the configuration at C-10, by its synthesis from toxol (4) as follows. Acetylation gave toxyl acetate(9), which on careful reduction with $NaBH_4$ gave the hydroxyacetate 10, which, in turn, was ethylated with ethyl iodide and silver oxide to give 11, and finally saponification of the latter gave synthetic toxethol (7) identical by NMR and IR spectra with the natural material and having a similar plain negative ORD curve. The structure

of 2,5-diacetylbenzofuran (5) was readily arrived at, when it was first isolated in 1963, by comparison of its ^{1}H NMR and IR spectra with those of samples prepared by oxidation of dehydrotremetone (3), toxol (4) and toxol acetate (9) and it was also synthesized from synthetic tremetone (1), prepared according to Bonner (36), by hydroxylation of the double bond and oxidative cleavage to give the diketone (38) and finally dehydrogenation with 5% Pd on C. We were surprised to find that samples of isolated dehydrotremetone (3) on standing, under normal conditions in vials in the laboratory, were oxidized to 2,5-diacetylbenzofuran (5). This surprising result may arise from dehydrotremetone acting as its own photosensitizer leading to a light catalyzed (2+2) cycloaddition of singlet oxygen to the isopropenyl double bond of dehydrotremetone and finally cleavage of this four membered intermediate to give 2,5-diacetylbenzofuran and formaldehyde.

we have tabulated the ^{1}H NMR spectral data on the benzofurans isolated from I. Wrightii and we have tabulated the ^{13}C NMR data on these compounds. These ^{1}H NMR data of these benzofurans are consistent with that reported by Bohlmann et al (13-21) for related structures and together provide a rather complete set of data for these naturally occurring benzofurans. The ^{13}C NMR data is the first such report. We have tabulated the major fragments observed in the mass spectra of the isolated benzofurans. In every case, except that of toxol, the parent ion or $M^{+}-CH_3$ is the base peak or at least a very large peak. The MS of toxol is thus ususual and we are uncertain as to what the peaks at m/e 187 and 185 are due to. By contrast the base peak (m/e 200) in toxyl angelate (6) corresponds to the loss of angelic acid as expected.

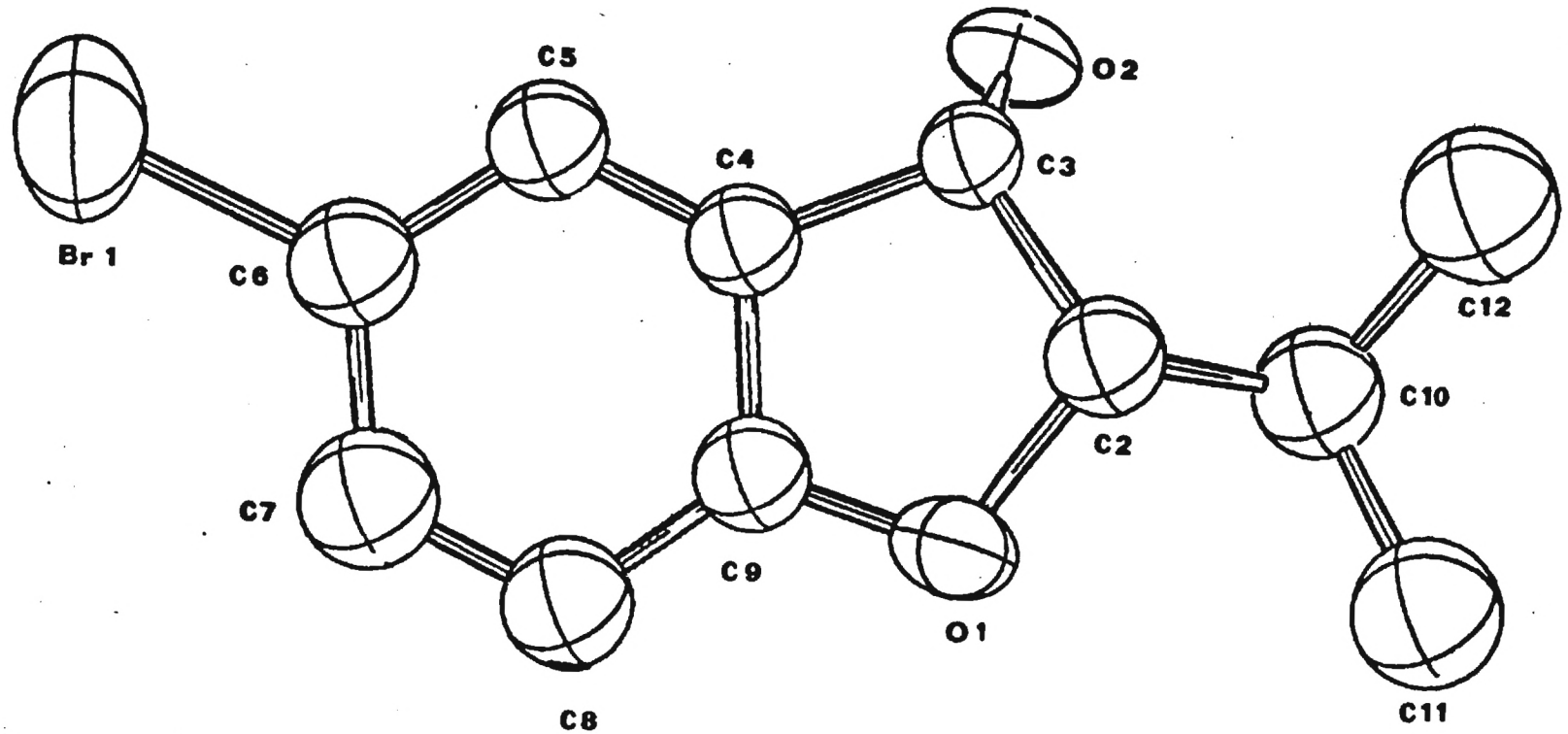
Toxol (4) was originally assigned to 2S,3S configuration and, as mentioned previously, the configurational assignment at C-2 was correlated with several compounds of known absolute configuration while the assignment at C-3 was

based on a single experimental observation, namely, the ozonolysis of toxol to yield, supposedly, (+) tartaric acid (38). A number of years ago we reported the syntheses of racemic trans and cis-2-isopropyl-3-hydroxy-5-acetyl-2,3-dihydrobenzofuran (37). The isomer which was spectrally identical with dihydrotoxol was assigned a cis relationship at C-2, C-3 based on the above mentioned ozonolysis of toxol to (+) tartaric acid. This assignment led to an unexpected consequence, namely, in synthetic dihydrotoxol and all of its precursors, the coupling constant for the vicinal C-2, C-3 protons was consistently smaller (J = 3-4.5 vs 5-6 Hz) than in the isomeric trans series in apparent violation of the Karplus equation. This anomalism led us to reinvestigate the configuration of toxol at C-3. Earlier (39) we presented, in a preliminary report, the conclusion that the absolute configuration of toxol was in fact 2S, 3R and therefore trans rather than cis as previously assumed and there was therefore no violation of the Karplus equation. This preliminary report was based on the X-ray analysis of the synthetic 2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrotoxol (m.p. 112-113°)(15) belonging to the series not related to toxol. At that time (1972) the X-ray data was not well refined. We have now repeated the X-ray analysis using more sophisticated means and the result is shown in Figure 1.

We have tabulated the C-2, C-3 vicinal proton NMR coupling constants of the various synthetic intermediate cis and trans dihydrobenzofurans prepared in our laboratory in the synthesis of dihydrotoxol (37), the natural products toxol (4), toxyl angelate (6) and toxethol (7) and their derivatives (9), (10) and (11). Unfortunately in the case of toxethol (7) the chemical shift of one of the olefinic protons overlapped that of the C-2 proton while the other olefinic proton overlapped that of the C-3 proton in a way that we could not unequivocally determine $J_{2,3}$ from our spectra. However, we

FIGURE 1

Computer Generated Picture of cis-2-Isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran



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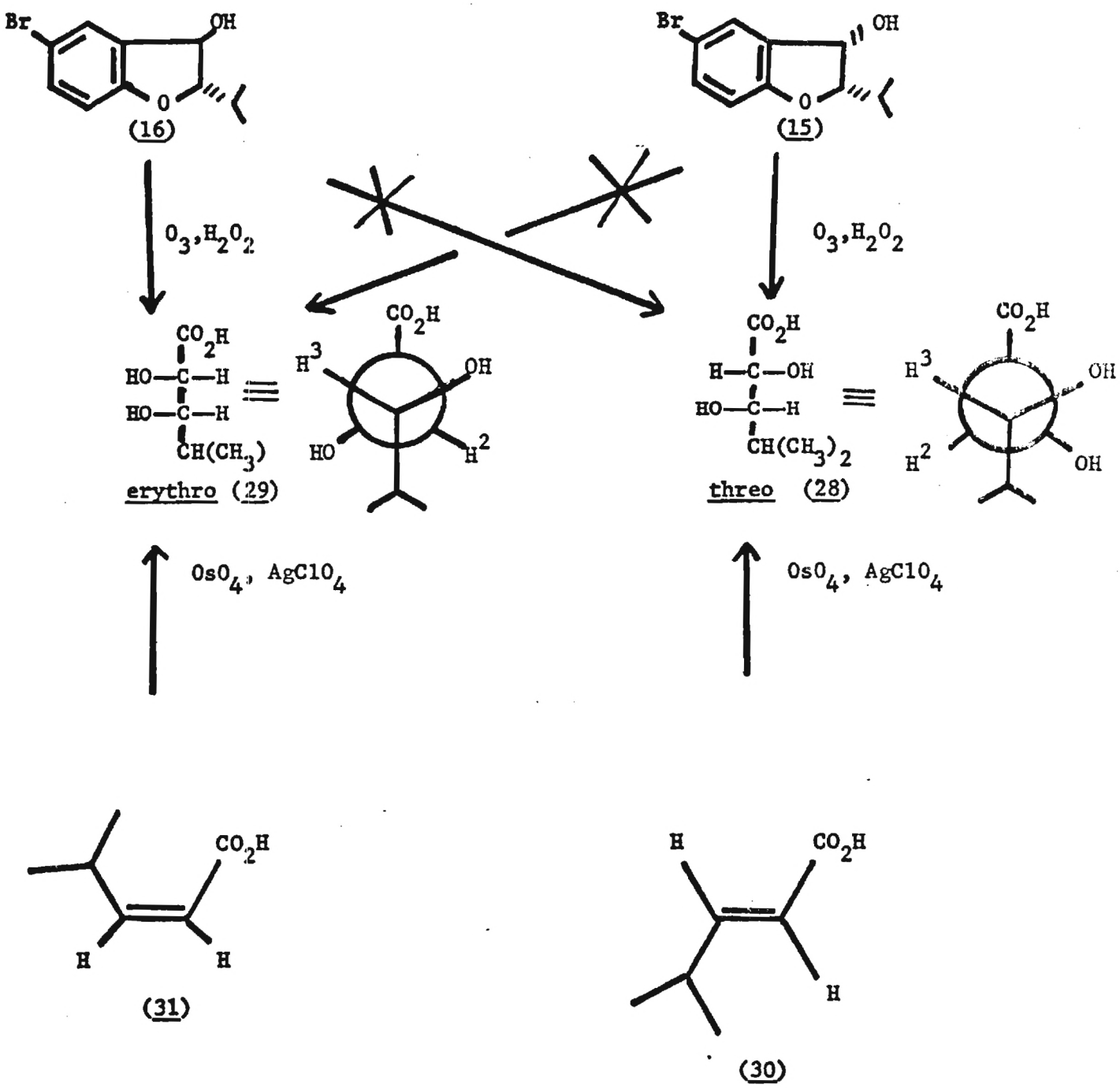
could estimate a maximum value of $J_{2,3}$~3 Hz and the corresponding acetate (11) and derivative (10) clearly showed values of $J_{2,3}$ of 3 Hz. We also prepared a series of 2-alkyl-3-hydroxy-5-bromo-2,3-dihydrobenzofurans (22-27). All of the cis and trans isomers are clearly distinguishable in each case by their differences in $J_{2,3}$ and the natural products toxol (4), toxyl angelate (6), and toxethol (7), on this basis, are trans.

Chemical support was obtained for the stereochemical assignments of the synthetic isomeric racemic 2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofurans 15 and 16 as follows (see Figure 2). Ozonolysis of the isomer of m.p. 112-113°(15) yielded threo-2,3-dihydroxy-4-methylpentanoic acid (29) while ozonolysis of the isomer of m.p. 445-45°(16) gave the corresponding erythro acid (29). The NMR spectra of the crude ozonolysis products from isomeric 15 and 16 clearly showed that in the former case only threo 28 was produced while in the latter case only erythro 29 was formed by adding the isomer not produced in the oxonolysis to the NMR tube to show that it in fact could be detected in small amounts. G.L.C. analysis also showed that each ozonolysis produced a single acid product. The authentic samples of racemic threo (28) and erythro-2,3-dihydroxy-4-methylpentanoic (29) acids were prepared by stereospecific cis hydroxylation of trans-4-methyl-2-pentenoic acid (30) and cis-4-methyl-2-pentenoic acid (31) respectively as illustrated in Figure 2. While threo and erythro 2,3-dihydroxy-4-methylpentanoic acids were prepared by stereospecific syntheses, their NMR spectra further confirmed the configurational assignments. Thus, the averaged spectrum (in D_2O) of 28 (preferred conformer indicated in Figure 2) showed $J_{2,3}$ = 2 Hz while that of 29 (preferred conformer indicated in Figure 2) showed $J_{2,3}$ = 5.5 Hz at room temperature as expected for the threo and erythro isomers respectively.

Figure 2

Relative Configurations of the 2-Isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofurans



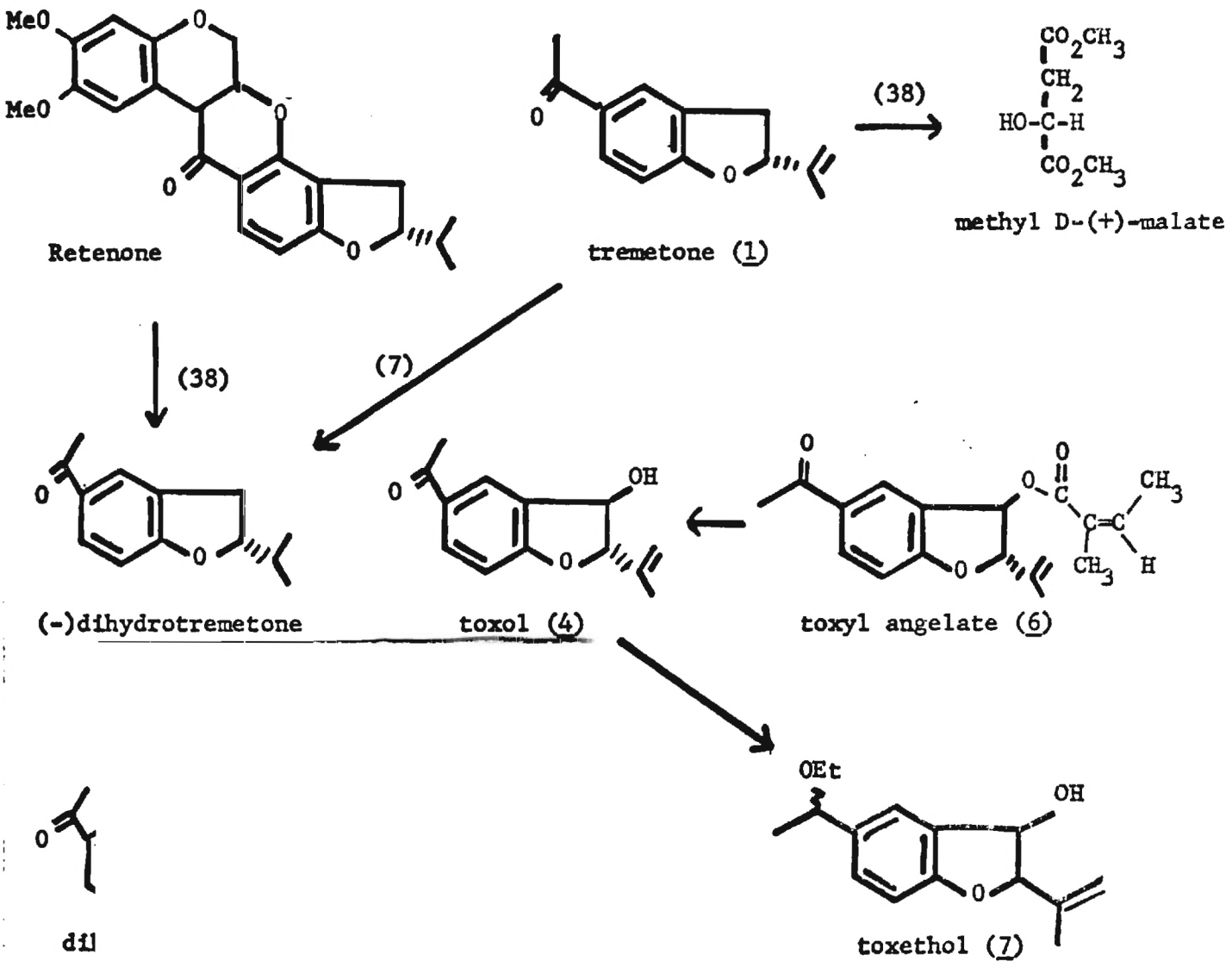
The one final inconsistency in this story of the configuration of toxol and related substances at C-3 remains the reported isolation of (+) tartaric acid in the ozonolysis of toxol (38). We have now repeated this experiment, but due to the short supply of natural toxol, we began with the more abundant toxyl angelate which was hydrolyzed to give toxol which, in turn, was ozonized. This time, we isolated only meso-tartaric acid as expected! Figure 3 summarizes the absolute configurational relationship of toxol and its congeners.

II. The Lower Terpenes

The ^{1}H nmr spectra of isocomene (12) and modhephene (13) revealed that both substances possessed two quaternary methyl groups, a methyl group attached to a tertiary carbon, a methyl group attached to a trisubstituted double bond and an olefinic hydrogen. In both the nmr spectra isocomene and modhephene the two quaternary methyl groups appeared as singlets and in the case of isocomene this turned out to be misleading as will be evident. The ^{13}C nmr spectra of both isocomene and modhephene verified that each contained a trisubstituted double bond and, in addition, revealed that each structure possessed three quaternary carbon atoms. The mass spectra of the two unknowns confirmed the molecular weights and elemental compositions deduced from the elemental analyses, but the base peak in isocomene (m/e 189) seemed to correspond to the loss of propylene ($M^+ - C_3H_6$) while in modhephene (m/e 162) it appeared to arise simply by the loss of a methyl group ($M^+ - CH_3$). Thus, while the similarities in the ^{1}H nmr spectra suggested the two substances were skeletally related, the great differences in the ^{13}C nmr and mass spectra showed that isocomene and modhephene possessed different skeleta. Both substances were converted into major diols by treatment with osmium tetroxide in pyridine and the ^{1}H nmr spectrum of the major diol obtained from isocomene clearly showed three methyl singlets, in addition to a

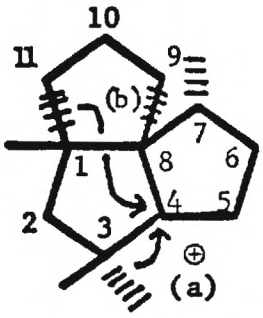
FIGURE 3

The Absolute Configuration of Toxol and its Congeners



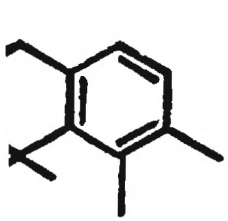
methyl doublet, thus revealing that none of the quaternary carbons in isocomene bore a gem dimethyl group whereas the ^1H nmr spectrum of the major diol from modhephene showed a six proton methyl singlet analogous to that shown in modhephene, suggesting that one of the quaternary carbons in modhephene did bare a gem dimethyl group. We were only able to arrive at the unequivocal structures (12) and (13) for these two unknowns by single crystal X-ray analyses of the above mentioned diols (43,44).

In the preparation of the cis diols of isocomene and modhephene, respectively, which were used for the above mentioned X-ray analyses, cis hydroxylation from one side predominated in each case. Thus, in the case of isocomene, the predominant isomer was formed by cis hydroxylation from the α side of (12) (cis to the two cis methyl groups), while in the case of modhephene, the predominant isomer was formed by α hydroxylation of (13) (cis to the unsubstituted bridge). A cursory examination of models of (12) and (13) fails to reveal any apparent steric or conformational preference for either face of the double bonds in these highly symmetrical tricycloalkenes and this remains an intriguing question. Another interesting observation is the difference in the magnitudes of the plain negative ORD curves with the molecular rotation curve of isocomene being about ten times greater than that of modhephene. An examination of models of the two molecules clearly reveals that modhephene (13) is the more symmetrical of the two and removal of the secondary methyl group would convert it into a non-chiral substance with a plane of symmetry running through the three carbon bridge bearing the gem dimethyl group and double bond. Since caryophyllene is by far the major sesquiterpenoid component of this plant, it is tempting to suggest it as a precursor to isocomene (12) and modhephene (13) It seems very likely that both these sesquiterpenes arise from the common intermediate carbonium ion indicated via methyl migration (path a) to give isocomene (12) or migration of bond C(1)-C(11)



to give modhephene (path b). The relative configurations of these two sesquiterpenes are consistent with this postulation.

Steam distillation of the hexane extract of the entire above ground portion of the plant yielded a yellow essential oil which upon distillation gave a low boiling fraction containing (+) limonene, (-) borneol, (-) carvone and bornyl acetate, which were isolated by chromatography on alumina. Chromatography of the higher boiling residue on silica gel yielded in some of the hexane eluents, a homogeneous (glc) colorless liquid in 2% yield based on steam volatile oil. The analytical and spectral data suggested that this unknown possessed one of



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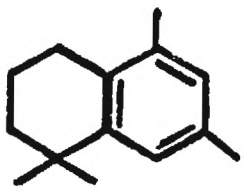

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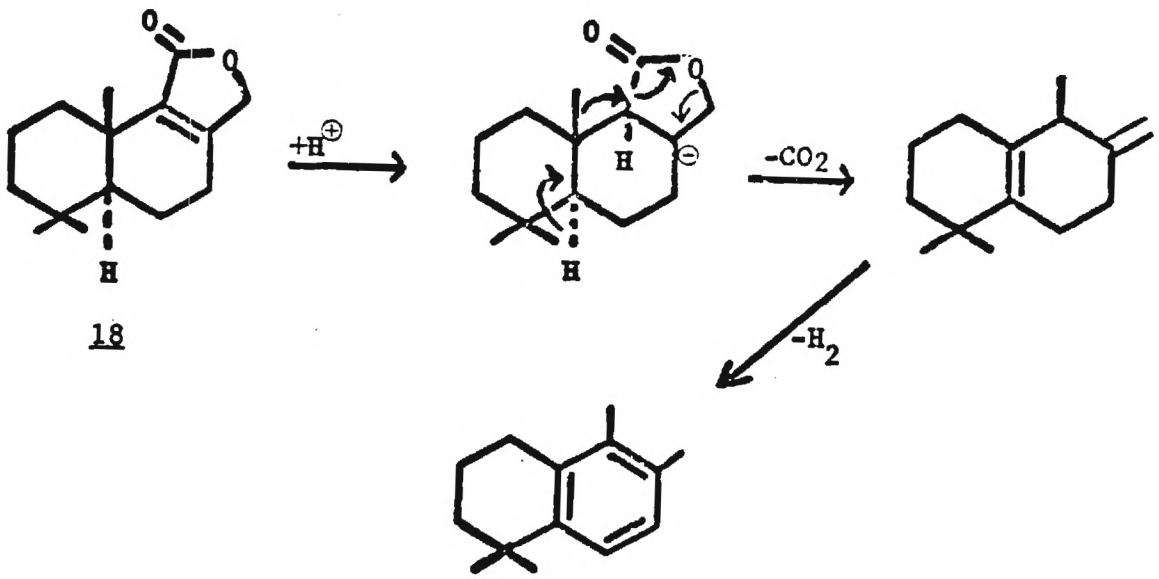
:trical isomer 4 was undertaken beginning with
:ion with succinic anhydride to give 4(2,5-
:id, followed by Huang-Minlon reduction to
.d, then esterification with diazomethane,
:ard reagent methylmagnesium iodide to give
pentanol, and finally Friedel-Crafts alkylation

with polyphosphoric acid to give 1,1,5,8-tetramethyl-1,2,3,4-tetrahydronaphthalene (15). The p-xylene used in the synthesis contained a small amount of m-xylene which reacted in a parallel series of reactions to give ultimately 1,1,5,7-tetramethyl-1,2,3,4-tetrahydronaphthalene (17) The two tetrahydronaphthalenes 15 and 17 were separated by chromatography on silica gel impregnated with silver nitrate, with 17 being eluted first. Tetralin 17 was identified by comparison of its ir and nmr spectra with those of an authentic sample prepared in a similar manner. While the ir and mass spectra of synthetic 15 were similar to those of the unknown, the two differed in glc retention time and the differences in their pmr spectra were particularly instructive. A comparison of the nmr spectra of 17 with that of 15 and the unknown suggested that the correct structure of the unknown was, in fact 14 and not 16 because in both the unknown and in 17 the gem dimethyl group and the two aromatic methyl groups had almost identical chemical shifts respectively, whereas in 15 both the gem dimethyl group and the aromatic methyl group at C-8 showed rather considerable deshielding as would be expected if the correct structure of the unknown were 16.

A search of the literature revealed that 1,2,3,4-tetrahydro-1,1,5,6-tetramethylnaphthalene (14) had recently been reported as a rearranged degradation product of the sesquiterpene avarol formed on dehydrogenation with 10% Pd-C at 270°. Indeed, the reported spectral properties and a copy of the nmr spectrum verified that our unknown was identical to the above mentioned degradation product. An examination of the carbon skeleton of 1,2,3,4-tetrahydro-1,1,5,6-tetramethylnaphthalene (14) indicates that it is a nordrimane sesquiterpene with the bridgehead methyl group missing. Indeed, a mechanistically feasible pathway for its biogenesis can be visualized from the known sesquiterpene isodremenin (18) as outlined. The extremely mild isolation procedure utilized, involving hexane extraction, steam distillation, fractional distillation and

finally chromatography on alumina make it highly unlikely that 14 is an artefact and, indeed, we know of no precursors, including isodremenin (18) which would lead to 14 under these conditions.



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Saponification of the methanolic plant extract gave an ether soluble dark red oil (1% based on dried plant) which on steam distillation gave an essential oil which upon further fractional distillation gave (-) carvone, (-) borneol and (-) caryophyllene. Chromatography of the fraction bp 75-95°/0.05 mm on alumina (act III) gave (-) caryophyllene oxide. Each of these terpenes was identical, within experimental error, by ir, nmr and $[\alpha]_D$ with authentic samples. When the methanolic plant extract was diluted with an equal volume of water then extracted with pentane, (+) limonene was obtained, identical by ir, nmr and $[\alpha]_D$ with an authentic sample.

Screening

The $CHCl_3$ partitions of the ethanol extracts of both Pecos Valley I. Wrightii and Rio Grande Valley I. Wrightii have been screened by NCI using the PS tumor system and found to be active but rescreening of old extracts showed a decrease in activity. Among the isolated benzofurans, slight activity was found in this tumor system for dehydrotremetone and toxyl angelate while toxol and 2,5-diacetylbenzofuran showed no activity and tremetone was only KB screened where it did not show activity. 7-[(3-methyl-2-butenyl)oxyl]courmarin also showed very slight PS activity. All of these compounds need to be provided to NCI in larger amounts for more extensive screening because the above screening was usually done at very low dose level. It is our opinion that we will find the really active compound in the aqueous-methanol layer from which all of the above mentioned compounds were isolated because it is from this fraction that NCI has found most of its active compounds and some of the above mentioned compounds already show some activity.. The proposed in-house KB screen will facilitate the location of the active compound among the large number of compounds known to be present in this fraction.

Staffing:*

Mr. J. P. Novak, Jr., graduate research assistant, July 1, 1976-June 30, 1977.

Mr. R. N. Harris, III, graduate research assistant, July 1, 1976-8/31/79

Mr. B. A. Ekpo, graduate research assistant, July 1, 1976 - 1/1/79

Dr. M. Ghosal, postdoctoral fellow, Sept. 1, 1976-May 31, 1977.

Mr. J. T. Baxter, graduate research assistant, Oct. 1, 1976-Aug. 1977.

Dr. C. Dickinson, postdoctoral fellow, June 1, 1978 - 8/31/79

Mr. P. May, graduate research assistant, July 1, 1977 - 7/1/78

* The research workers indicated were not necessarily paid in full from this grant but devoted the time period indicated to this project.

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Publications

(1) "The Benzofurans of Isocoma Wrightii. Structure and Stereochemistry." L. H. Zalkow, C. Dickinson, B. A. Ekpo, L. T. Gelbaum, R. N. Harris, III, E. Keinan, J. R. Novak, Jr., C. T. Ramming and D. van Derveer, J. Nat. Products (Lloydia), 42, 203 (1979).

(2) "The Lower Terpenoids of Isocoma Wrightii." L. H. Zalkow, R. N. Harris, III and N. I. Burke, J. Nat. Products (Lloydia), 42, 96 (1979)

(3) "Modhephene: A Sesquiterpenoid Carbocyclic [3.3.3]Propellane. X-ray Crystal Structure of the Corresponding Diol." L. H. Zalkow, R. N. Harris, III, and D. van Derveer, Chem. Comm. (London), 420 (1978).

(4) "Isocomene: A Novel Sesquiterpene from Isocoma Wrightii. X-ray Crystal Structure of the Corresponding Diol." L. H. Zalkow, R. N. Harris, III, D. van Derveer and J. A. Bertrand, Chem. Comm. (London), 456 (1977).

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Reprinted from JOURNAL OF NATURAL PRODUCTS, Vol. 42, No. 2, Mar-Apr, 1979

THE BENZOFURANS OF *ISOCOMA WRIGHTII*.[1-3] STRUCTURE AND STEREOCHEMISTRY.

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ABSTRACT.—Fractionation and chromatography of the anti-tumor chloroform portion of the ethanol extract of *Isocoma wrightii* led to the isolation of the family of benzofurans: toxol (2S, 3R-2-isopropenyl-3-hydroxy-5-acetyl-2,3-dihydrobenzofuran) (**4**); toxyl angelate (**6**); tremetone (2S-isopropenyl-5-acetyl-2,3-dihydrobenzofuran) (**1**); dehydrotremetone (**3**); 2,5-diacetylbenzofuran (**5**) and toxethol [2S-isopropenyl-3R-hydroxy-5-1(1'-ethoxyethyl)-2,3-dihydrobenzofuran] (**7**). In addition, a number of steroids, triterpenes, sesquiterpenes, monoterpenes, and hydrocarbons were isolated. The relative and absolute configurations of the natural 2,3-dihydrobenzofurans have been determined by chemical correlations, nmr analysis of the C–2, C–3 proton coupling constants, and an X-ray analysis of racemic *cis*-2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran. The isomeric racemic *trans*-2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran was converted into racemic dihydrotoxol. Racemic *cis* and *trans* 2-methyl, 2-ethyl, and 2-n-propyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran were synthesized and their ^{1}H nmr spectra, particularly the C–2, C–3 coupling constants, are discussed. Toxethol was synthesized from toxol, and saponification of toxyl angelate gave toxol. Ozonolysis of *cis* and *trans*-2-isoproyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran gave, respectively, *threo* and *erythro*-2,3-dihydroxy-4-methylpentanoic acid, which were stereo-specifically synthesized.

"Milk sickness," a disease dreaded in the nineteenth century, was shown to be due to the animal's ingestion of *Eupatorium rugosum* and *Isocoma wrightii* (1, 2). The toxic principle was reported to be tremetol, an unsaturated alcohol (2–5). It was later found (6) that tremetol was not a pure substance but a mixture composed of a sterol fraction and a ketone fraction (7). Only the ketone fraction gave Couch's (5) characteristic sulfuric acid color test for "tremetol." The ketone fraction was separated into three new benzofurans: tremetone (**1**), hydroxytremetone (**2**), and dehydrotremetone (**3**). At about the same time, we reinvestigated "rayless goldenrod tremetol" (8) and isolated from it a ketone fraction containing dehydrotremetone (**3**) and a new benzofuran, toxol (**4**). The isolation of "tremetol" involves an intensive alkaline saponification, and we have now found that the non-saponified hexane and alcoholic extracts of rayless goldenrod contain the related benzofurans 2,5-diacetylbenzofuran (**5**), toxyl angelate (**6**), and toxethol (**7**).[6] Careful chromatographic techniques have revealed that rayless goldenrod also contains tremetone (**1**).

[1]This plant was formerly known as *Haplopappus* (*Aplopappus*) *heterophyllus*. See D. S. Correll and M. C. Johnston, "Manual of Vascular Plants of Texas," Texas Research Foundation, Renner, Texas, 1970.

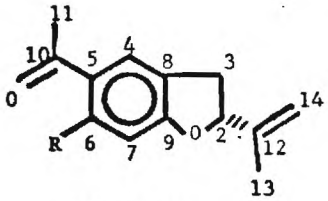
[2]Preliminary accounts of some of this work were presented as follows: L. H. Zalkow, J. R. Novak, Jr., B. Ekpo and R. N. Harris, III, 28th Southeast Regional Meeting, American Chemical Society, Gatlinburg, Tenn., Oct. 27–29, 1976. L. H. Zalkow, J. R. Novak, Jr., B. Ekpo and R. N. Harris, III, 18th Annual Meeting, American Society of Pharmacognosy, Seattle, Washington, August 11–13, 1977.

[3]A portion of this work appeared in a preliminary communication (39).

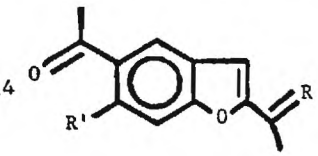
[4]To whom inquiries should be made.

[5]Taken in part from: J. R. Novak, Jr., Ph.D. Dissertation, Georgia Institute of Technology, Atlanta, Georgia, 1977; E. Keinan, Master's Thesis, Ben-Gurion University of the Negev, Beer-Sheva, Israel, 1972; L. T. Ramming, Master's Thesis, Oklahoma State University, Stillwater, Oklahoma, 1963.

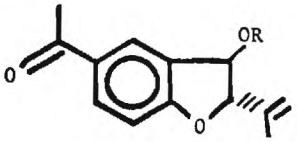
[6]We have coined the trivial name toxethol for **7** to maintain consistency with the trivial name toxol for **4**.



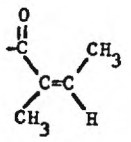
(1) R=H
(2) R=OH



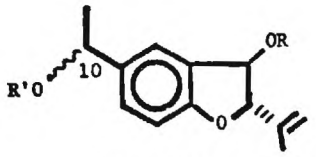
(3) R=CH_2, R'=H
(5) R=O, R'=H
(8) R=CH_2, R'=OH



(4) R=H
(6) R=



(9) R=$COCH_3$



15 16

(7) R=H, R'=CH_2CH_3
(10) R=$COCH_3$, R'=H
(11) R=$COCH_3$, R'=CH_2CH_3

While none of the above-mentioned benzofurans have been implicated as the causative agents of "trembles" in higher animals, some of them show biological activity. Toxol (4) and dehydrotremetone (3) are bacteriostitic against a number of bacteria (8); and tremetone (1), dehydrotremetone (3), and hydroxytremetone (2) are toxic to goldfish (7). A recent investigation showed that "white snakeroot tremetol" causes ketoacidosis in chicks (9). The chloroform fraction of the chloroform-water partition of the ethanol extract of rayless goldenrod shows antitumor activity against P388 lymphocytic leukemia tumors.[7] Toxol (4) and toxyl angelate (6) show weak antitumor activity in this system.[7]

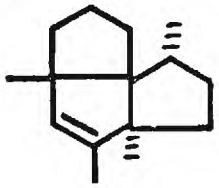
Until recently, benzofurans comprised a very small group of natural products. The first member of this group to be reported was euparin (8), (6-hydroxydehydrotremetone) from *Eupatorium purpurem* (10). A recent compendium of natural products (11) classified these simple benzofurans into two separate groups: the C_6C_2 compounds (shikimate derived) i.e. tremetone (1), dehydrotremetone (3), and toxol (4) and the phloroglucinols (polyketide derived) i.e. euparin (8) and hydroxytremetone (2). This division appears to be unjustified since the only biosynthetic study of this family thus far reported has shown that the acetophenone moiety of dehydrotremetone was derived from acetate via the polyacetate pathway (12). As expected, the furan ring and its side chain were formed from an isoprenoid compound. Recently, benzofurans of this type were found to occur more commonly than previously suspected in the Compositae family, and approximately forty such compounds are now known (13–31).

Synthesis of the benzofurans such as dehydrotremetone (3) which contain a double bond at C-2, C-3 has not posed much difficulty (32–34). Synthesis of 2,3-dihydrobenzofurans such as tremetone (1) also seems to be readily accom-

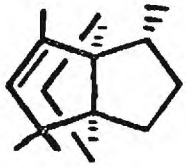
[7]Screening by NCI of the National Institutes of Health.

plished (35, 36). On the other hand, synthesis of 2,3-dihydrobenzofurans containing an oxygen substituent at C–3, such as toxol (**4**), has not appeared in the literature. However, the closely related *cis* and *trans* dihydrotoxols have been synthesized (37). Relative stereochemical assignments in the 2-oxygenated benzofurans of the toxol (**4**) group have been largely based on examination of the C–2, C–3 proton coupling constants in their nmr spectra (13–31). The absolute configuration of tremetone (**1**) and toxol (**4**) at C–2 is based on chemical correlations with rotenone and methyl D(+) malate (38), while the absolute configuration of toxol (**4**) at C–3 is based on an X-ray analysis and a chemical degradation of a synthetic intermediate (39). Further details of these stereochemical assignments are discussed later in this paper. No other independent absolute configurational studies of this family of benzofurans have been reported.

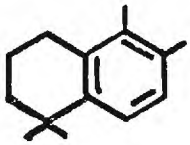
Rayless goldenrod (*I. wrightii*) has been an unusually rich source of secondary plant metabolites of diverse structures. Thus, in addition to the above mentioned benzofurans, the novel steroids 5α-androstane-3β,16α,17α-triol (40) and stigmasta-8(14),22-dien-3β-ol (41) were isolated, and we have now identified stigmasta-5,22-dien-3β-ol and stigmasta-8(14)-en-3β-ol. From the hexane extract, we have identified the triterpenes friedelin, friedelan-3α-ol(42), friedelan-3β-ol, and squalene and the diterpene phytol. In the sesquiterpene family we have found the relatively common β-caryophyllene and β-caryophyllene oxide and the highly unusual isocomene (**12**) (43), modhephene (**13**) (44), and the tetrahydronaphthalene **14** (45).



(**12**)



(**13**)



(**14**)

Isocomene and modhephene represent new sesquiterpenoid skeleta, while **14** is a nordrimane sesquiterpene (C_{14}). Among the monoterpenes, we have identified (+) limonene, (−) carvone, (−) borneol, and bornyl acetate (45). The hexane extract of rayless goldenrod yielded a complex hydrocarbon fraction. After chromatography nonacosane, henitriacontane, and tritriacontane were isolated, and several others were tentatively identified by glc. Among the fatty acids, stearic acid was isolated. Hexanoic, octanoic, lauric, myristic, palmitic, and linoleic acid were identified by glc of their methyl esters. Finally, a family of fatty alcohols has been isolated but not yet completely characterized.

DISCUSSION

The benzofurans dehydrotremetone (**3**), toxyl angelate (**6**), 2,5-diacetylbenzofuran (**5**), toxethol (**7**), and toxol (**4**) were isolated, in the order given, by chromatography of the acid-free aqueous methanol fraction on acid-washed alumina (activity grade III). This fraction was obtained as follows: The dried leaves and flowers of *I. wrightii* were defatted with hexane, then extracted with 95% ethanol. The latter extract, after removal of solvent, was partitioned between chloroform and water. The chloroform fraction, after removal of solvent, was further partitioned between aqueous methanol (1:9) and hexane. The aqueous methanol fraction, after removal of the solvent, was dissolved in ether. This

solution was washed with cold 5% sodium hydroxide solution. Glc indicated, in addition to the above mentioned benzofurans (**3–7**), the presence of tremetone (**1**). Tremetone was isolated by the following procedure. The ethanol extract of the above-ground plant material, after initial defatting with hexane, was saponified with potassium hydroxide (~10%) in aqueous methanol (1:1). The ether-soluble portion of this mixture was a "red jelly" analogous to the original rayless goldenrod "tremetol" of Couch (5).

This "red jelly" was separated into ketone and non-ketone fractions using Girard's T reagent. Glc of the ketone fraction showed three major components of increasing retention time in the ratios of 1.3:1:5.3. These components were isolated by chromatography on silica gel and identified as dehydrotremetone (**3**), tremetone (**1**) and toxol (**4**), respectively. Toxyl angelate (**6**) was most readily isolated from the original hexane extract of the above-ground plant material as follows. The hexane extract was steam distilled and the ether-soluble nonvolatile residue was distributed between benzene and aqueous ethanol. The benzene fraction was washed with a cold 5% sodium hydroxide solution and chromatographed on alumina (activity grade II) and then silica gel to give toxyl angelate (**6**).

The structure of toxyl angelate (**6**) was surmised from analysis of ^{1}H and ^{13}C nmr spectra and its mass spectrum (see tables 1, 2 and 3) and confirmed by its

TABLE 1. *^{1}H-Nmr data of the isolated benzofurans.*[a]

C	(3)	(5)	(1)	(4)	(6)	(7)
2	—	—	5.27 (t, 9.0)	5.05 (d, 3.7)	5.00 (d, 2.5)	5.09 (m)
3	6.67 (s)	7.57 (s)	a) 3.08 (m) b) 3.36 (m)	a) 4.97 (b) b) 3.87 (b) (OH)	6.17 (d, 2.5)	4.88 (m)
4	8.17 (d, 2.0)	8.35 (d, 1.7)	7.84 (m)	8.02 (d, 2.0)	8.04 (d, 2.0)	7.32 (m)
5	—	—	—	—	—	—
6	7.93 (dd, 2.0, 8.7)	8.13 (dd, 1.7, 9.0)	7.84 (m)	7.88 (dd, 2.0, 8.5)	7.93 (dd, 2.0, 8.5)	7.24 (dd, 2.0, 8.5)
7	7.43 (d, 8.7)	7.62 (d, 9.0)	6.81 (d, 9.0)	6.88 (d, 8.5)	6.89 (d, 8.5)	6.84 (d, 8.5)
8	—	—	—	—	—	—
9	—	—	—	—	—	—
10	—	—	—	—	—	4.40 (q, 6.5)
11	2.22 (s)	2.68 (s)	2.53 (s)	2.51 (s)	2.47 (s)	1.40 (d, 6.5)
12	—	—	—	—	—	—
13	1.90 (s)	2.63 (s)	1.76 (bs)	1.73 (s)	1.71 (s)	1.76 (s)
14	a) 5.23 (bs) b) 5.83 (bs)	—	a) 5.09 (bs) b) 5.18 (bs)	a) 4.93 (bs) b) 5.08 (bs)	a) 4.90 (d, 1.0) b) 5.03 (d, 1.0) R=angeloyl 1.92 (dq, 7.3, 1.5) 6.06 (qq, 7.3, 1.5) 1.77 (dq, 1.5, 1.5)	a) 5.09 (m) b) 4.88 (m) 15) 3.32 (q, 7.0) 16) 1.17 (t, 7.0)

[a]Spectra were run at 99.95 MHz in $CDCl_3$ solution using a JEOL PFT-100 instrument in FT mode; chemical shift values are expressed in δ values (PPM) relative to TMS.

hydrolysis to the known toxol (**4**). Likewise, the structure of toxethol (**7**), partially suggested from its spectral properties, was firmly established, except for the configuration at C-10, by its synthesis from toxol (**4**) as follows. Acetylation gave toxyl acetate (**9**), which on careful reduction with $NaBH_4$ gave the hydroxyacetate **10**. In turn, **10** was ethylated with ethyl iodide and silver oxide to give **11**. Finally, saponification of the latter gave synthetic toxethol (**7**) identical by nmr and ir spectra with the natural material and having a similar plain negative ord curve. The structure of 2,5-diacetylbenzofuran (**5**) was readily arrived at when it was first isolated in 1963 by comparison of its ^{1}H nmr and ir spectra with those

TABLE 2. *^{13}C-Nmr data of the isolated benzofurans.*[a]

C	(3)	(5)	(4)	(6)	(1)	(7)
2	156.4 (s)[c]	152.8 (s)	94.3 (d)	90.9 (d)	86.5	93.4 (d)
3	110.2 (d)	113.1 (d)	75.3 (d)	76.3 (d)	33.9	76.2[b]
4	124.5 (d)[b]	127.5 (d)[b]	131.7 (d)[b]	132.5 (d)[b]	130.3[b]	122.6 (d)[d]
5	131.6 (s)	126.4 (s)	128.5 (s)	126.6 (s)	126.9	127.5 (s)[c]
6	121.4 (d)[b]	124.3 (d)[b]	126.3 (d)[b]	127.6 (d)[b]	124.8[b]	128.0 (d)[d]
7	102.5 (d)	111.8 (d)	109.4 (d)	109.5 (d)	108.3	109.3 (d)
8	128.4 (s)	132.8 (s)	130.1 (s)	130.8 (s)	129.9	136.2 (s)[c]
9	157.5 (s)[c]	156.7 (s)	163.5 (s)	163.9 (s)	163.3	158.6 (s)
10	196.3 (s)	195.4 (s)	196.3 (s)	194.8 (s)	195.3	77.1[b]
11	26.4 (q)	26.5 (q)[c]	26.2 (q)	26.1 (q)	26.1	23.9 (q)
12	131.9 (s)	186.7 (s)	140.5 (s)	139.6 (s)	142.6	141.1 (s)
13	19.0 (q)	26.3 (q)[c]	17.4 (q)	17.6 (q)	17.0	15.2[e]
14	113.6 (t)	———	112.2 (t)	113.1 (t)	111.9	111.6 (t)
					R = Angeloyl	15) 63.3 (t)
					166.3 (s)	16) 17.4[e]
					124.7 (s)	
					20.3 (q)	
					138.8 (d)	
					15.8 (q)	

[a]Spectra were run at 25 MHz in $CDCl_3$ solution using a JEOL PFT-100 instrument in FT mode; chemical shift values are expressed in δ values (PPM) relative to TMS. b, c, d, e, assignments may be reversed.

of samples prepared by oxidation of dehydrotremetone (**3**), toxol (**4**), and toxol acetate (**9**). It was also synthesized from synthetic tremetone (**1**) prepared according to Bonner (36) by hydroxylation of the double bond and oxidative cleavage to give the diketone (38) and, finally, dehydrogenation with 5% Pd on carbon. We were surprised to find that samples of isolated dehydrotremetone (**3**) standing under normal conditions in vials in the laboratory were oxidized to 2,5-diacetylbenzofuran (**5**). This surprising result may arise from dehydrotremetone's acting as its own photosensitizer leading to a light-catalyzed (2+2) cycloaddition of singlet oxygen to the isopropenyl double bond of dehydrotremetone and, finally, cleavage of this four-membered intermediate to give 2,5-diacetylbenzofuran and formaldehyde.

TABLE 3. *Major peaks from the mass spectra of the isolated benzofurans.*

Compound	M^+ *m/e*	Misc.	$[M^+-CH_3]$	$[M^+-(CH_3-C\equiv O)]$	$[CH_3-C\equiv O^+]$
(3)	200 (52%)		*m/e* 185 (100%)	*m/e* 157 (40%)	*m/e* 43 (31%)
(5)	202 (41%)		*m/e* 187 (100%)	*m/e* 159 (17%)	*m/e* 43 (14%)
(1)	202 (91%)		*m/e* 187 (53%)	*m/e* 159 (50%)	*m/e* 43 (100%)
(4)	218 (28%)	*m/e* % 187 55 185 43 163 34 162 50	*m/e* 203 (32%)	*m/e* 175 (16%)	*m/e* 43 (100%)
(6)	300 (3%)	*m/e* 200 (3)[⊕] 100% *m/e* 83 angeloyl 90%	*m/e* 200-CH_3 *m/e* 185 (80%)	*m/e* 200-CH_3-C≡O *m/e* 157 (19%)	*m/e* 43 (58%)
(7)	248 (20%)	*m/e* 215 (24%)	*m/e* (200) (100%)		

In table 1 we have tabulated the ^1H nmr spectral data on the benzofurans isolated from *I. wrightii*, and in table 2 we have tabulated the ^{13}C nmr data on these compounds. These ^1H data of these benzofurans are consistent with those reported by Bohlmann *et al.* (13–21) for related structures and, together, provide a rather complete set of data for these naturally-occurring benzofurans. The ^{13}C nmr data in table 2 is the first such report. In table 3 we have tabulated the major fragments observed in the mass spectra of the isolated benzofurans. In every case, except that of toxol, the parent ion or M^+-CH_3 is the base peak or, at least, a very large peak. The ms of toxol is thus unusual, and we are uncertain of the cause of the peaks at *m/e* 187 and 185. The base peak (*m/e* 200) in toxyl angelate (**6**) corresponds to the loss of angelic acid, as expected.

Toxol (**4**) was originally assigned to 2S,3S configuration and, as mentioned previously, the configurational assignment at C–2 was correlated with several compounds of known absolute configuration. The assignment at C–3 was based on a single experimental observation, namely, the ozonolysis of toxol to yield, supposedly, (+) tartaric acid (38). A number of years ago we reported the synthesis of racemic *trans* and *cis*-2-isopropyl-3-hydroxy-5-acetyl-2,3-dihydrobenzofuran (37). The isomer which was spectrally identical with dihydrotoxol was assigned a *cis* relationship at C–2, C–3 based on the above-mentioned ozonolysis of toxol to (+) tartaric acid. This assignment led to an unexpected consequence. In synthetic dihydrotoxol and all of its precursors, the coupling constant for the vicinal C–2, C–3 protons was consistently smaller (J=3–4.5 vs 5–6 Hz) than in the isomeric *trans* series, in apparent violation of the Karplus equation. This anomaly led us to reinvestigate the configuration of toxol at C–3. In a preliminary report (39), we presented the conclusion that the absolute configuration of toxol was in fact *2S, 3R* and, therefore, *trans* rather than *cis* as previously assumed, and there was, therefore, no violation of the Karplus equation. This preliminary report was based on the X-ray analysis of the synthetic 2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrotoxol (mp 112–113°) (**15**) belonging to the series not related to toxol. At that time (1972), the X-ray data was not well refined. We have now repeated the X-ray analysis using more sophisticated means as follows.

Epoxy cement was used to mount a crystal of (**15**) with the approximate dimensions .45 x .45 x .25 mm on a glass fiber so that the longest crystal dimension was approximately parallel to the fiber axis. The crystal was coated with epoxy cement since an unprotected crystal decomposed in the atmosphere. Unit cell parameters and the orientation matrix were determined on a Syntex $P2_1$ four circle diffractometer equipped with a graphite monochromator using MoKα radiation. Unit cell parameters obtained were a=12.808(8)Å, α=104.35(5)°, andV= 1862(a)Å. The calculated density of 1.38 g cm^{-1} for six formula units per unit cell agrees with the experimental density of 1.36 g cm^{-1} measured by the flotation method using aqueous zinc chloride. The crystal belonged to the rhombohedral system, and space group $R\bar{3}$ (No. 148) (46) was assumed. Successful refinement of the structure confirmed this choice. Intensity data were collected using θ–2θ scans with X-ray source and monochromator settings identical to those used for the determination of the unit cell parameters. From a total of 2121 unique reflections collected out to 2θ=50°, 793 were accepted as statistically above background on the basis that F was greater than 5 σ(F). Computations were performed using standard programs (47). For structure factor calculations the scattering factors were taken from Cromer and Mann's tabulation (48). The agreement factors were defined in the usual way as

$$R = (\Sigma||F_o|-|F_c||)/(\Sigma|F_o|)$$

and

$$R_w = [\Sigma(|F_o|-|F_c|)(w^{1/2})/\Sigma(|F_o|)(w^{1/2})]$$

In all least-squares refinements, the quantity minimized was $w(|F_o|-|F_c|)^2$. A weighting scheme based on counting statistics $w = 1.00/(\sigma(F)^2 + .01\ F^2)$ was employed for calculating R_w in the least-squares refinement. The structure was solved using the automatic centrosymmetric direct methods section of SHELX-76. The E-map generated by this program contained all non-hydrogen atoms except the isopropyl group. The carbons in the isopropyl group were located from a subsequent difference Fourier synthesis. Hydrogen positions were calculated using the capabilities of the SHELX-76 program. Carbon-hydrogen bond lengths were fixed at 1.08Å, the hydrogen temperature factors were varied, and the hydrogen positions were recalculated before each cycle of refinement. The final parameters varied included an overall scale factor, positional parameters for the oxygens and carbons, anisotropic thermal parameters for the oxygens, and bromine and isotropic thermal parameters for the carbons and hydrogens (80 variables; 793 observations). The final R factor was .102 and $R_w = .113$, full-matrix least-squares refinement.

Table 4 shows the coordinates of the oxygen, carbon, and hydrogen atoms. Figure 1 shows a computer-generated picture of the molecule, clearly indicating that this material (m.p. 112–113°) is *cis*-2-isopropyl-3-hydroxy-5-bromo-2,3-di-

TABLE 4. *Coordinates of the atoms (standard deviations are indicated in parenthesis and refer to the last decimal place).*

Atom	X	Y	Z	U11	U22	U33	U23	U13	U12
BR	.8897(2)	.3537(3)	.9364(2)	.108(2)	.194(3)	.075(2)	.026(2)	.015(1)	.063(2)
01	.4298(8)	.1167(8)	.5878(8)	.069(7)	.064(6)	.072(7)	.032(5)	.039(6)	.025(5)
02	.5565(8)	.3269(8)	.5136(8)	.087(7)	.068(6)	.078(7)	.049(6)	.055(6)	.046(6)
Atom	**X**	**Y**	**Z**	**U**					
C2	.453(1)	.126(1)	.483(1)	.063(4)					
HC2	.465(1)	.053(1)	.431(1)	.09(5)					
C3	.503(1)	.219(1)	.518(1)	.047(3)					
HC3	.611(1)	.198(1)	.462(1)	.04(3)					
C4	.615(1)	.227(1)	.642(1)	.047(3)					
C5	.719(1)	.282(1)	.715(1)	.059(4)					
HC5	.784(1)	.330(1)	.689(1)	.05(3)					
C6	.744(1)	.278(1)	.829(1)	.069(4)					
C7	.650(2)	.214(2)	.855(2)	.085(6)					
HC7	.667(2)	.209(2)	.941(2)	.5(2)					
C8	.544(1)	.162(1)	.780(1)	.070(4)					
HC8	.474(1)	.117(1)	.802(1)	.06(4)					
C9	.532(1)	.168(1)	.673(1)	.055(3)					
C10	.350(1)	.131(2)	.401(1)	.079(5)					
HC10	.334(1)	.209(2)	.439(1)	.08(4)					
C11	.374(2)	.129(2)	.289(1)	.087(6)					
HC11	.301(2)	.131(2)	.228(1)	.16(5)					
HC11	.392(2)	.051(2)	.256(1)	.16(5)					
HC11	.447(2)	.201(2)	.306(1)	.16(5)					
C12	.241(2)	.035(2)	.376(2)	.103(6)					
HC12	.224(2)	.037(2)	.455(2)	.23(7)					
HC12	.251(2)	−.046(2)	.339(2)	.23(7)					
HC12	.170(2)	.045(2)	.318(2)	.23(7)					

The form of the thermal ellipsoid expression is $\exp[-2\pi^2(U_{11}h^2a^{*2}+U_{22}k^2b^{*2}+U_{33}l^2c^{*2}-2U_{12}hka^*b^*+2U_{13}hla^*c^*+2U_{23}klb^*c^*)]$.

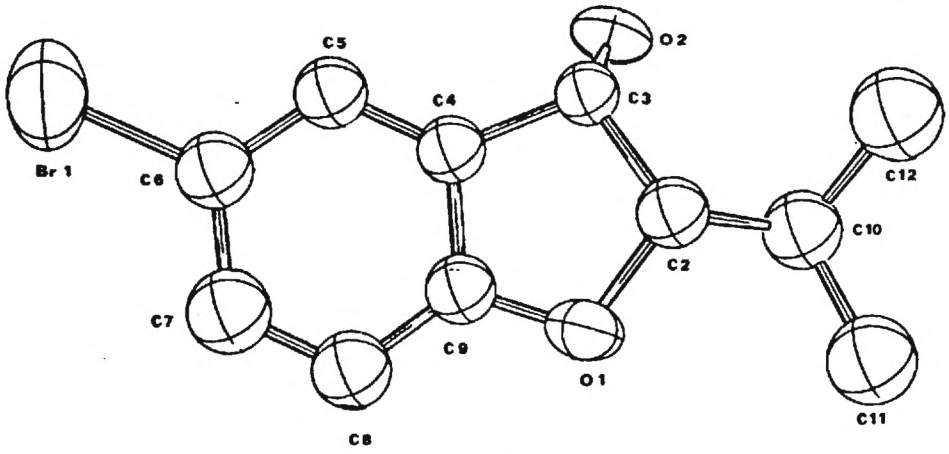
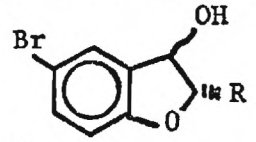


FIG. 1

hydrobenzofuran. Therefore, the isomeric substance (mp 44.5–45°) which was converted into dihydrotoxol must be *trans*-2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran. Dihydrotoxol, toxol, toxol angelate and toxethol must be *trans* at C–2, C–3 and have the absolute configurations indicated in **4**, **6** and **7** since the absolute configuration at C–2 in toxol is well established (38).

In table 5 we have tabulated the C–2, C–3 vicinal proton nmr coupling constants of the various synthetic intermediate *cis* and *trans* dihydrobenzofurans prepared in our laboratory in the synthesis of dihydrotoxol (37), the natural pro-

TABLE 5. *C-2, C-3 Vicinal ^1H coupling constants.*

Compound	$J_{2,3}$ Hz		Ref.
	Cis	Trans	
			
R=Br, R'=H	(15) 5.5	(16) 4.2	(37)
R=Br, R=$COCH_3$	(17) 6	(18) 3.5	(37)
R=CO_2H, R'=H	—	(19) 4.5	(37)
R=$COCH_3$, R'=H	(20) 6	(21) 4	(37)
			
R=Me	(22) 6.5	(23) 3.5	
R=Et	(24) 6.0	(25) 3.5	
R=n-C_3H_7	(26) 5.5	(27) 3.7	
toxol (4)	—	3.7	
toxyl acetate (9)	—	3	
toxyl angelate (6)	—	2.5	
toxethol (7)	—	~3	
(10)	—	3	
(11)	—	3	

ducts toxol (**4**), toxyl angelate (**6**) and toxethol (**7**), and their derivatives (**9**), (**10**) and (**11**). Unfortunately in the case of toxethol (**7**) the chemical shift of one of the olefinic protons overlapped that of the C-2 proton, while the other olefinic proton overlapped that of the C-3 proton in a way that we could not unequivocally determine J_{213} from our spectra. However, we could estimate a maximum value of $J_{213}\sim 3$ Hz, and the corresponding acetate (**11**) and derivative (**10**) clearly showed values of J_{213} of 3 Hz. We also prepared a series of 2-alkyl-3-hydroxy-5-bromo-2,3-dihydrobenzofurans (**22**–**27**). As can be seen from table 5, all of the *cis* and *trans* isomers are clearly distinguishable in each case by their differences in J_{213}. On this basis the natural products toxol (**4**), toxyl angelate (**6**) and toxethol (**7**) were determined to be *trans*.

Chemical support was obtained for the stereochemical assignments of the synthetic isomeric racemic 2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofurans **15** and **16** as follows (see figure 2). Ozonolysis of the isomer of mp 112–113° (**15**) yielded *threo*-2,3-dihydroxy-4-methylpentanoic acid (**28**), whereas ozonolysis of the isomer of mp 44.5–45° (**16**) gave the corresponding *erythro* acid (**29**). The nmr spectra of the crude ozonolysis products from isomeric **15** and **16** clearly showed that in the former case only *threo* **28** was produced while in the latter case

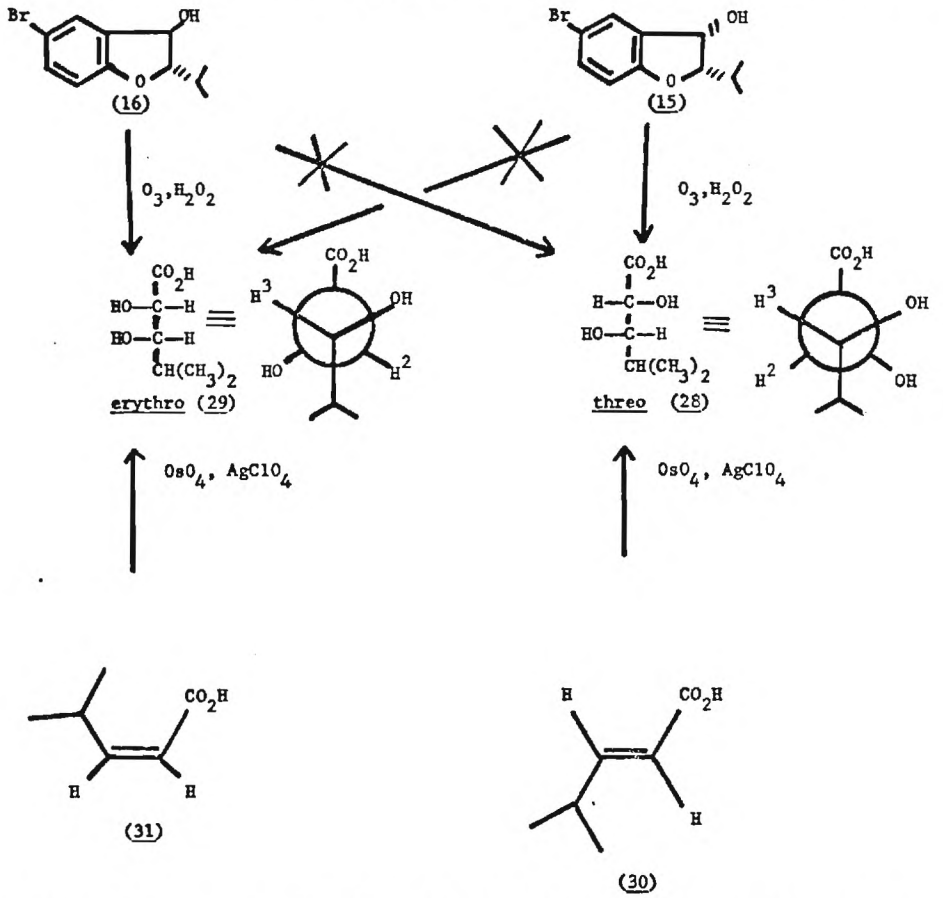


FIG. 2. *Relative configurations of the 2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofurans.*

only *erythro* **29** was formed by adding the isomer not produced in the ozonolysis to the nmr tube to show that it could be detected in small amounts. Glc analysis also showed that each ozonolysis produced a single acid product. The authentic samples of racemic *threo* (**28**) and *erythro*-2,3-dihydroxy-4-methylpentanoic (**29**) acids were prepared by stereospecific *cis* hydroxylation of trans-4-methyl-2-pentenoic acid (**30**) and *cis*-4-methyl-2-pentenoic acid (**31**), respectively, as illustrated in figure 2. While *threo* and *erythro* 2,3-dihydroxy-4-methylpentanoic acids

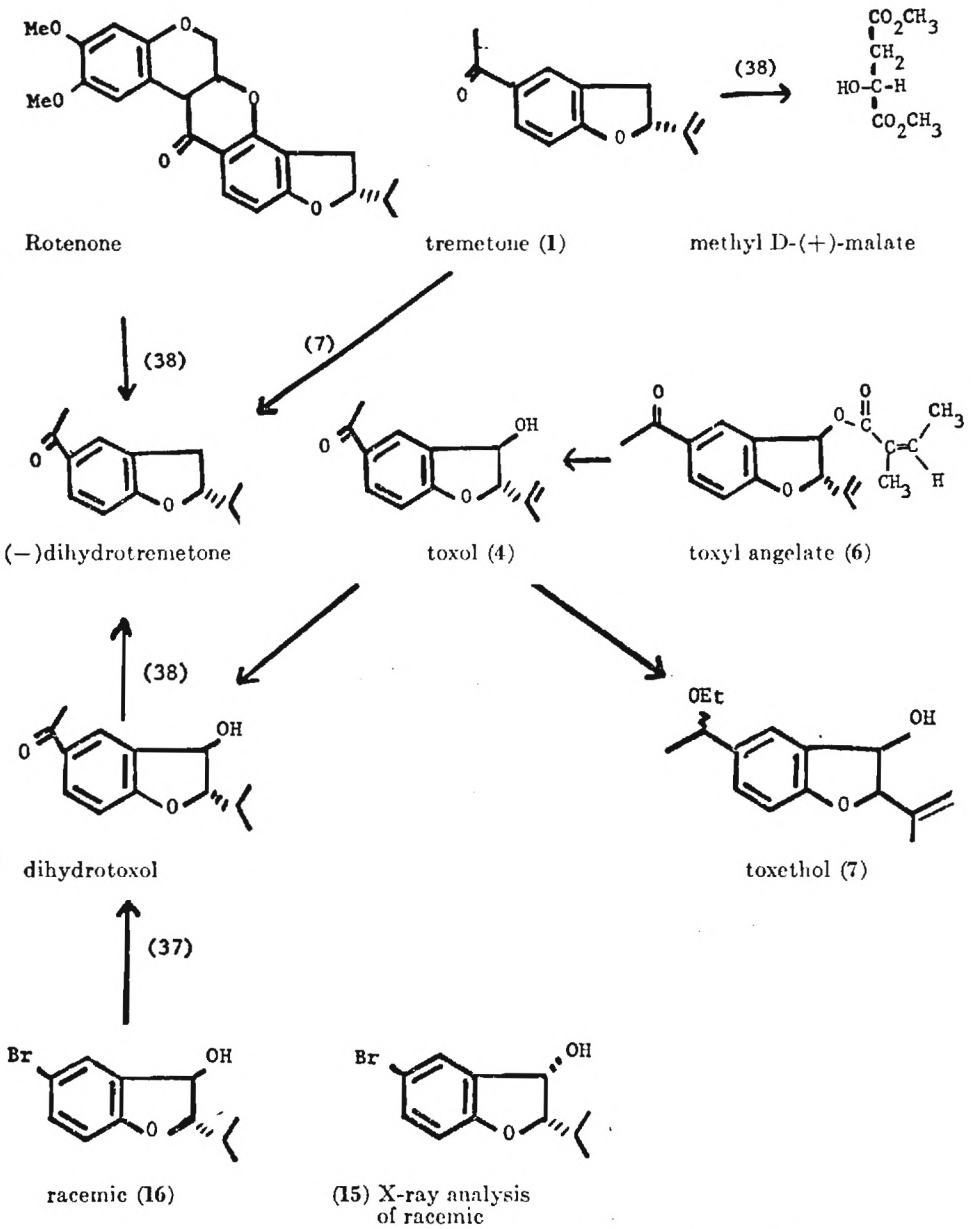


FIG. 3. *The absolute configuration of toxol and its congeners.*

were prepared by stereospecific syntheses, their nmr spectra further confirmed the configurational assignments. Thus, the averaged spectrum (in D_2O) of **28** (preferred conformer indicated in figure 2) showed $J_{2,3}=2$ Hz while that of **29** (preferred conformer indicated in figure 2) showed $J_{2,3}=5.5$ Hz at room temperature as expected for the *threo* and *erythro* isomers, respectively.

The one final inconsistency in this story of the configuration of toxol and related substances at C-3 remains the reported isolation of (+) tartaric acid in the ozonolysis of toxol (38). We recently attempted to repeat this experiment, but, due to the short supply of natural toxol, we began with the more abundant toxyl angelate (2.5 g), which was hydrolyzed to give toxol (1.8 g). The toxol was then ozonized as previously described (38). This time, we could detect no tartaric acid in the ozonolysis product based on nmr and hplc analysis by comparison with an authentic sample. In addition, we could not detect the presence of any of the possible precursor 2-keto-3,4-dihydroxypentanoic acid. Thus, we must consider the original report of the formation of (+) tartaric acid as an unexplained erroneous result. Figure 3 summarizes the absolute configurational relationship of toxol and its congeners.

EXPERIMENTAL[8]

ISOLATION OF DEHYDROTREMETONE (**3**), TOXYL ANGELATE (**6**), 2,5-DIACETYLBENZOFURAN (**5**), TOXETHOL (**7**), AND TOXOL (**4**).—The above-ground parts of rayless goldenrod (*Isocoma wrightii* (Gray) Rydb.) were collected in the vicinity of Artesia, New Mexico, in October 1968. The material was air dried, ground, and stored in sealed bags until extracted in 1974. Twelve kilograms of the plant material were extracted with 19 liters of hexane in Soxhlets for 2 days. Removal of the hexane with a rotary evaporator gave 250 g of extract (fraction A). Extraction of the marc in a similar manner with 95% ethanol gave 275 g of ethanol extract (fraction B). Fraction B was partitioned between chloroform and water (2 liters each). The chloroform layer was washed with a saturated brine solution then dried over anhydrous sodium sulfate. The chloroform was then removed *in vacuo* to give a greenish-brown tar residue (205 g) designated fraction C. Fraction C was partitioned between two liters each of hexane and aqueous methanol (1:9). The hexane layer was concentrated to a green syrup (35 g) and designated fraction D. The aqueous methanol layer was concentrated to 0.5 liter and then one liter of water was added. After the addition of salt to prevent an emulsion, the mixture was extracted with two liters of chloroform. The chloroform extractive was dried over anhydrous sodium sulfate, filtered and then evaporated *in vacuo* to give 163 g of a dark brown tar designated fraction E. Fraction E was dissolved in 1.5 liters of ether, and the resulting solution was extracted with three 300 ml portions of ice cold 5% sodium hydroxide solution and then with a like amount of distilled water. The ether solution was dried over anhydrous sodium sulfate and filtered. After the ether was removed, there remained a viscous greenish-brown syrup (23.5 g) (fraction F). Glc (6', 5% SE-30 column at 175°) indicated fraction F contained dihydrotremetone (**3**), toxyl angelate (**6**), 2,5-diacetylbenzofuran (**5**), toxethol (**7**), toxol (**4**), possibly tremetone (**1**), and a number of unknown constituents.

Chromatography of fraction F (22.4 g) on 1680 g of acid-washed alumina (activity grade III) gave in the hexane-benzene (2:3) eluent, first, 0.57 g dehydrotremetone (**3**) and then 0.28 g toxyl angelate (**6**) (physical and spectral properties are given below). Elution with hexane-ether (1:1) gave 1.1 g of a red-brown solid. The glc of this solid showed a 1:1 ratio of 2,5-diacetylbenzofuran (**5**) and toxethol (**7**). These two substances (**5** and **7**) were separated by further chromatography on 100 g of acid-washed alumina (activity grade II). Elution with chloroform gave 0.32 g of >95%pure 2,5-diacetylbenzofuran (**5**). Further elution with chloroform gave 0.31 g of toxethol (**7**). Elution of the original column of acid-washed alumina (activity grade III) with chloroform-ethyl acetate (9:1) gave 4.65 g of a brown oil. The glc of the oil indicated two components in a ratio of 85:15. On rechromatography of this fraction on 500 g acid-washed alumina (activity grade II), both components were again eluted together with hexane-ethyl acetate (4:1). After the minor, more volatile, component was removed by short path distillation at 110–120°/0.18 mm, almost pure (90-95% by glc) toxol (**4**) remained.

[8]M.p.'s were taken on a Kofler hot stage and are uncorrected. Ir spectra were recorded with a Perkin Elmer 237 B spectrophotometer. ^{1}H nmr spectra were obtained with a Varian A-60D or T60 spectrometer, except where otherwise indicated, with Me_4Si as an internal standard (δ 0); ^{13}C nmr spectra were run on a JOEL-PFT-100 Ft spectrometer. Mass spectra were run on a Hitachi RMV-7 spectrometer; gas chromatography was done with a F&M Biomedical Gas Chromatograph, model 402; and ord spectra were recorded using a Jasco ORD/UV-5 instrument.

DEHYDROTREMETONE (3).—Mp 84–85° (from hexane); rpt. mp 87.5–88.5° (7); glc R_t=5.3 min, 6' x ¼" 5% SE-30 glass column at 170°; νKBr 2955, 2925, 1675, 1595, 1565, 1370, 1310, 1275, 1245, 1165, 925, 840, 820 cm^{-1}; λ95% EtOH 254 (4.57), 282 (4.26), 285 (4.22), 294 (4.15), 310 nm (log ε3.67); ^1H nmr-see table 1; ^{13}C nmr-see table 2; mass spec-see table 3.

TOXYL ANGELATE (6).—Bp 120–130°/0.1 mm (air bath), sample purified by hplc on a size B EM Reagents silica gel 60 column; Rf 0.86, tlc on Eastman chromatogram 13181 silica gel sheet developed with ethyl acetate-hexane (1:1); glc R_t=24 min, 6' x ¼" 5% SE 30 column at 170°; Calc. for $C_{15}H_{20}O_4$: C, 71.98, H, 6.71. Found: C, 72.38, 72.31, 72.25, H, 7.08, 7.07, 7.09; νfilm 1715, 1680, 1645, 1610, 1360, 1295, 1265, 1230, 1150 cm^{-1}; ord (C, 2.31; $CHCl_3$): $[\phi]_{589}=-311.7$, $[\phi]_{550}=-363.6$, $[\phi]_{500}=-467.3$, $[\phi]_{450}=-675.3$, $[\phi]_{400}=-961.0$, $[\phi]_{350}=-1506.5$; $[\alpha]^{25}D-114.7$ (C, 4.62, $CHCl_3$), by polarimeter; ^1H nmr-see table 1; ^{13}C nmr-see table 2; mass spec-see table 3.

Saponification of toxyl angelate with 8% ethanolic potassium hydroxide gave toxol in the neutral fraction, identical by glc, ir, nmr, ord with an authentic sample.

2,5-DIACETYLBENZOFURAN (5).—Mp 139–140° (from MeOH), rpt mp 139–10° (8); glc R_t= 6.6 min (same conditions as listed for dehydrotremetone); $\nu CHCl_3$ 1680, 1630, 1570, 1365, 1305, 1280, 1160 cm^{-1}; λEtOH 253 (4.61), 286 nm (log ε4.28); ^1H nmr-see table 1. ^{13}C nmr-see table 2. Mass spectrum-see table 3.

2,5-Diacetylbenzofuran was synthesized as follows. Salicylaldehyde was converted into 2-acetylbenzofuran, as previously described (49): yield 45%, mp 70–71°, rpt. mp 72–73° (49). Treatment of 2-acetylbenzofuran with methyl magnesium iodide, as described by Bonner *et al.* (35), yielded 2-benzofuryl-2-propanol, which was then hydrogenated with W-2 Raney nickel catalyst to give 2-(2,3-dihydrobenzofuryl)-2-propanol. The latter was acylated with acetic acid and trifuoroacetic anhydride to give 2-(2,3-dihydro-5-acetylbenzofuryl)-2-propyl acetate, mp 95°, rpt. mp 95–96° (35), in overall yield of 30%. The latter acetate was pyrolyzed at 330° according to Bonner's procedure (36) to give racemic tremetone in quantitative yield. Synthetic racemic tremetone was converted, as previously described (38), into 2,5-diacetyl-2,3-dihydrobenzofuran with 40% yield. A mixture of 0.176 g of 2,5-diacetyl-2,3-dihydrobenzofuran and 0.35 g of 5% Pd on carbon was sealed, under vacuum, in a heavy walled tube which was heated at 215–220° for 1.5 hr. After cooling, the contents of the tube were dissolved in acetone, and the catalyst was removed by filtration. Evaporation gave 0.085 g (50% yield) of a solid which, after recrystallization from methanol, was identical with natural 2,5-diacetylbenzofuran.

TOXETHOL (7).—Bp 110–125°/0.1 mm (air bath); R_t=5.5 min, 6' x ¼" 3% OV-17 glass column at 175°; Calc. for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.25; H, 8.52; νfilm 3375, 1615, 1485, 1240, 1100, 1065, 1020, 900, 820, 760 cm^{-1}; λ95% EtOH 291 (3.31), 285 (3.36), 229 nm (log ε 3.78); ord (C, 1.32; $CHCl_3$): $[\phi]_{589}=-47°$, $[\phi]_{550}=-66°$, $[\phi]_{500}=-94°$, $[\phi]_{450}=-132°$, $[\phi]_{400}=-207°$, $[\phi]_{350}=-443°$; ^1H nmr-see table 1; ^{13}C-see table 2; mass spec-see table 3.

Toxethol (7) was synthesized from toxol (4) as follows. Toxol acetate (9) was prepared from toxol (4), as previously described, (8) with 96% yield. It had the following physical properties: νfilm 1740, 1680, 1610, 1225–1275 (br); δ($CDCl_3$): 1.77 (3 H,s), 2.11 (3 H,s), 2.56 (3 H,s); mass spec *m/e* 260 (M^+, ~1%), 200 (33%), 185 (44%), 157 (16%), 43 (100%).

To 467 mg of toxol acetate in 5 ml of methanol at 0° was added 34 mg of $NaBH_4$. The reaction mixture was stirred at 0° for 1 hr and then worked up in the usual way. The nmr of the resulting 470 mg of a colorless oil indicated >95% of the desired hydroxyacetate **10**. Bp 125–130°/0.12 mm (air bath); glc R_t=4.3 min, 6' x ¼" 5% SE-30 column at 200°; νfilm 3400, 1740, 1615, 1490, 1235, 1020, 825 cm^{-1}; λ95% EtOH 292 (3.50), 285 (3.58), 226 nm (log ε 3.87); δ($CDCl_3$): 1.47 (d, 3 H, *J*=7 Hz), 1.75 (s, 3H), 2.10 (s, 3 H), 5.01 (m, 3 H), 6.13 (d, 1 H, *J*= 3 Hz); mass spec *m/e* 262 (M^+, 16%), 202 (61%), 187 (51%), 184 (48%), 43 (100%).

A mixture of freshly distilled ethyl iodide (2.5 ml), hydroxyacetate (50 gm), and silver oxide (132 mg) was stirred at 65° for 3 days. The mixture was cooled and chloroform (5 ml) was added. The solution was then filtered, washed successively with aqueous sodium thiosulfate and water, and dried over magnesium sulfate. When evaporated, this solution yielded a pale yellow oil (53 mg). The ^1H nmr of the oil indicated a mixture of desired ether **11** and starting material **10** in a ratio of 3:2. Chromatography on silica gel gave the desired **11** in the hexane-ether (13:2) eluent. It had the following physical properties: νfilm 1740, 1620, 1235, 1115, 1025, 825 cm^{-1}; δ($CDCl_3$) 1.18 (3 H, t, *J*=7 Hz), 1.41 (3 H, d, *J*=7 Hz), 1.79 (3 H, bs), 3.37 (2 H, q, *J*=7 Hz), 4.38 (1 H, q, *J*=7 Hz), 5.03 (1 H, bm), 6.18 (1 H, d, *J*=3 Hz); exact mass Calc. for $C_{17}H_{22}O_4$: 290.152, found: 290.153; *m/e* 290 (M^+, 1%), 230 (50%), 215 (91%), 185 (49%), 43 (100%).

Hydroxy acetate **11** (61 mg) was added to a solution of methanol (2 ml) and 3N sodium hydroxide (0.25 ml). The entire solution was heated at reflux for 1 hr, cooled to room temperature, diluted with brine (10 ml) and then extracted with ether. When washed with brine, dried over magnesium sulfate and evaporated, the solution yielded a pale yellow oil (52 mg); the ^1H nmr of the oil indicated it was ~95% pure toxethol (7). Distillation gave 50 mg of synthetic toxethol, bp 113–120°/0.12 mm (air bath). The ^1H nmr and ir spectra of the synthetic and natural toxethol were essentially indistinguishable and the mass spectra and ord curves of the two were similar but different in relative magnitudes. The latter differences could be

due to instrumental variation or, more probably, to the presence in the synthetic toxethol of the diastereomer, which differed from the natural isomer in configuration at the chiral center bearing the ethoxyl group.

TOXOL (4).—Mp 52–53° (from ether-petroleum ether); bp 110°/0.05 mm; glc R_t=8.6 min, 6' x ¼" 5% SE-30 glass column at 170°; $\nu CHCl_3$ 3450, 1670, 1660, 1610 cm^{-1}; ord (C, 2.41, EtOH): $[\phi]_{589}=-50.0°$, $[\phi]_{550}=-63.6°$, $[\phi]_{500}=-90.8°$, $[\phi]_{450}=-140.8°$, $[\phi]_{400}=254.4°$, $[\phi]_{360}=449.7°$; ^1H nmr=see table 1; ^{13}C nmr=see table 2; mass spec-see table 3.

PREPARATION OF THE "RED JELLY" (TREMETOL) AND ISOLATION OF TREMETONE (1).—The ethanol plant extract (193 g) fraction B, was dissolved in 1.5 liters of 50% aqueous methanol containing 105 g of potassium hydroxide. After the solution was refluxed for 48 hrs., most of the methanol was removed with the water aspirator. The resulting solution was diluted with 1 liter of water and then continuously extracted with ether for 48 hrs. Drying over anhydrous sodium sulfate and evaporation of the solvent gave 7.4 g of "red jelly." The "red jelly" (4.5 g) was dissolved in 50 ml of methanol containing 4 g of Girard's T Reagent and 1 ml of acetic acid. This solution was refluxed for 1 hr. After being cooled, the solution was poured into 10% sodium carbonate (50 ml) and then extracted with ether. After drying, the ether layer gave 3.6 g of the non-ketone fraction on evaporation. Acidification of the aqueous layer to pH2, extraction with ether, drying, and evaporation gave 0.9 g of the ketone fraction. Glc analysis (6' x ¼" 5% SE-30 glass column at 170°) showed three major components at R_t 5.3, 6.3, and 8.6 min in a ratio of 1.3:1:5.3. Chromatography of the ketone fraction on silica gel gave the compound of R_t=5.3 min in 10% ether in benzene eluent. It was identified as dehydrotremetone (3). Further elution with this solvent gave the component of R_t=6.3 min which was identified as tremetone (1) (see below for properties). Finally elution with 30% ether in benzene gave the component of R_t=8.6 min, which was identified as toxol.

TREMETONE (1).—Mp 38–39° (from ether-benzene); Calc. for $C_{13}H_{14}O_2$: C, 77.20; H, 6.98; Found: C, 77.15; H, 7.01; glc R_t=6.3 min (conditions above); νCCl_4 1672, 1605, 1265, 1230, 905 cm^{-1}; λEtOH 226 (3.94), 279 (3.90), 286 nm (log ϵ 3.90); ord (C, 0.89; EtOH): $[\phi]_{589}=-76°$, $[\phi]_{550}=-92°$, $[\phi]_{500}=-126°$, $[\phi]_{450}=-172°$, $[\phi]_{400}=-287°$, $[\phi]_{350}=-609°$; ^1H nmr-see table 1; ^{13}C nmr-see table 2; mass spec-see table 3.

THE NON-KETONE FRACTION. ISOLATION OF PHYTOL, HENTRIACONTANE, SQUALENE, STIGMASTA-8(14),22-DIEN-3β-OL, STIGMASTA-5,22-DIEN-3β-OL, STIGMASTA-8(14)-EN-3β-OL.—A temperature-programmed glc study of the non-ketone fraction of the "red jelly" indicated that it was a complex mixture separable into low and high boiling components. The non-ketone fraction (34 g) was chromatographed on Merck acid-washed alumina (activity grade I, 380 g). The benzene eluent (4.1 g) was shown by glc to contain predominantly one component. Rechromatography on Merck acid-washed alumina (activity grade I) gave phytol in the benzene eluent with the same spectral properties as previously described (50). Glc R_t=11.5 min (4' x ¼" 3% SE-30 on 100/120 Gas Chrom Q. Program of hold at 150° for 8 min, programmed to rise at 20°/min to 245° and hold at 245°); $\nu CHCl_3$ 3400, 2935, 1650, 1460, 1380, 980 cm^{-1}; $\delta(CDCl_3)$ 0.83 (3 H, d, J=4 Hz), 0.84 (6 H, s), 1.30 (18 H, b), 1.65 (3 H, s), 2.00 (4 H, m), 4.19 (2 H, bd), 5.43 (1 H, m); mass spec m/e 296 (M^+, 1%), 71 (100%).

The hexane eluent from the chromatography of the non-ketone fraction gave a 2 g fraction. Glc (column and program as mentioned above) of the fraction indicated the presence of three major components with R_t=15.6, 16.5 and 19 min, respectively. Rechromatography on Merck acid washed alumina (activity grade I) gave the two components of R_t 16.5 and 19 min in the first hexane eluent. Rechromatography on the same type of alumina (ratio of alumina to material 255:1) gave the component of longest R_t in one of the hexane eluents. A final chromatography with a ratio of alumina to substrate of 515:1 yielded this material analytically pure. It was identified as hentriacontane by the following spectral and physical properties (51): glc R_t=5.4 min (6' x ¼", 3% OV 17 glass column at 290°; mp 66–68°, rpt. mp 67° (52); νCCl_4 2950, 2930, 2850, 1460, 1255, 1095 and 1015 cm^{-1}; mass spec m/e 436 (M^+, 4%), 57 (100%). Mass spectral and glc studies were used to confirm the structure of hentriacontane ($C_{31}H_{64}$) using authentic samples (Applied Sciences Laboratory & Chem. Sample Co.) of triacontane ($C_{30}H_{62}$) and dotriacontane ($C_{32}H_{66}$). Use of mixed injections showed that hentriacontane fell between the two unknowns in R_t. The mass spectra comparisons clearly showed that the unknown was a straight chain hydrocarbon containing one more CH_2 group than triacontane and one less than dotriacontane.

Further elution with hexane from the column which yielded hentriacontane gave the component with the original R_t=15.6 min. After rechromatography on alumina, this material was obtained pure (glc) and was identified as squalene by comparison of its spectral properties with those reported (53). They were as follows: $\delta(CDCl_3)$: 1.60 (24 H, s, methyls), 1.98 (20 H, bs, methylenes), 5.10 (6 H, bm, olefin protons); mass spec: m/e 410 (M^+, 2%), 81 (100%).

THE STEROL FRACTION.—The chloroform eluent of the alumina chromatography of the original non-ketone fraction was found by glc to contain three major high molecular weight compounds (R_t=19.7, 20.4 and 21.4 min, 6' x ¼" 5% SE-30 column at 285°). Rechromatog-

raphy on Merck acid-washed alumina (100:1) gave, in the chloroform eluent, the unseparated three high molecular weight compounds free of low molecular weight contaminants. Further chromatography of the latter on (activity grade III) Merck acid-washed alumina (100:1) gave the component of R_t=20 min in the benzene eluent. It was identified as stigmast-8(14), 22-dien-3β-ol by the following physical and spectral properties (41): glc R_t=20.4 min (column above), 6.3 min (5' x ⅛", 1.5% OV-101 column at 285°); mp 164–165°, rpt mp 165–166 (41); $\nu CHCl_3$ 3600, 2950, 2865, 1460, 1375, 1145, 975 cm^{-1}; $\delta(CDCl_3)$ 0.55 (3 H, s), 0.80 (3 H, s), 3.62 (1 H, bm), 5.00 (2 H, m); mass spec 412 (M^+, 3%), 43 (100%).

The other two high molecular compounds were not separable by chromatography on alumina, silica gel, or silica gel impregnated with silver nitrate. A fraction which showed the three components by glc on the 5% SE-30 column mentioned above showed four components on a 6' x ⅜" 3% OV-17 column at 290° with R_t=14.0, 16.3, 18.4 and 20.1 min. The component with R_t=16.3 min was identified as the above-mentioned stigmasta-8(14),22-dien-3β-ol. Preparative glc on the last-mentioned column gave the components of R_t=14.0 min and 18.4 min, but the component of R_t=20.1 could not be obtained in sufficient quantity for good spectral analysis. The component of R_t=14 min was identified as stigmasta-5,22-dien-3β-ol by comparison with an authentic sample (Aldrich Chemical Co. 5440-9). It had the following physical properties: mp 168–170: mp of authentic sample 170°, mixed mp 168–170°; $\nu CHCl_3$ 3600, 2965, 2875, 1460, 1380, 1030, 974 cm^{-1}; $\delta(CDCl_3)$, 0.70 (3 H, s), 1.01 (3 H, s), 5.09 (2 H, b), 5.35 (1 H, b); *m/e* 412 (M^+, 17%), 55 (100%). The component of R_t=18.4 min was identical by physical and spectral properties to those reported for stigmasta-8(14)-en-3β-ol (54): mp 109–111°, rpt mp 108–111 (54); νCCl_4 3600, 2945, 2855, 1455, 1375, 1035 cm^{-1}; $\delta(CDCl_3)$ 0.54 (3 H, s), 0.80 (3 H, s); *m/e* 414 (100%), 255 (45%).

THE HEXANE EXTRACT. THE BENZOFURANS.—The original hexane extract of rayless golden rod contains a large variety of compounds including benzofurans, sesquiterpenes, triterpenes, sterols, hydrocarbons and long chain alcohols. In a future publication we will discuss this extract in more detail. We present here only the isolation of toxyl angelate and its congeners.

Steam distillation of the hexane extract (400 g) gave an ether soluble non-volatile fraction (360 g) which was partitioned between benzene, ethanol, and water (3:0.75:0.25). The benzene layer was washed with cold 5% sodium hydroxide solution to yield 90 g of a neutral fraction. Chromatography (20 g) on Merck neutral alumina (activity grade II) (50:1) gave, in the hexane-benzene eluent (1:1), 3.8 g of material. Concentration of the solvent precipitated a mixture of friedelin, friedelin-3α-ol, and friedelin-3β-ol. After chromatography on Merck neutral alumina (activity grade II) the mother liquor gave toxyl angelate, which was obtained analytically pure after hplc chromatography on silica gel. The hexane-benzene eluent (1:4) of the original column deposited crystalline 2,5-diacetylbenzofuran, while the chloroform eluent gave toxol.

THE MODIFIED COUCH PROCEDURE (5). ISOLATION OF 2,5-DIACETYLBENZOFURAN.—The ground plant material (6 kg) was extracted with 20 liters of methanol for 72 hr. Removal of the methanol with a water aspirator gave 1 kg of tarry residue, which was taken up in an equal volume of chloroform and then extracted with water. The solvent was removed from the chloroform portion. The resulting residue was dissolved in 50% aqueous ethanol. After filtration, the homogeneous solution was diluted with water until the solution was 30% ethanol. The latter solution was filtered and the filtrate evaporated to give 53 g of residue. This residue was taken up in benzene, and the benzene soluble portion was chromatographed on Merck acid-washed alumina (activity grade I). The ether-benzene (3:2) eluent deposited 4.5 g of 2,5-diacetylbenzofuran, mp 139–140°.

OZONOLYSIS OF *cis* AND *trans*-2-ISOPROPYL-3-HYDROXY-5-BROMO-2,3-DIHYDROBENZOFURAN.—A stream of ozone in oxygen (4.5%) was bubbled, at room temperature, through a solution prepared by dissolving 2 g of *cis*-2-isopropyl-3-hydroxy-5-bromo-2,3-dehydrobenzofuran (mp 105–106°; $J_{2,3}$ 5.5 Hz), prepared as previously described (37), in 30 ml of acetic acid for 24 hr. Hydrogen peroxide (8 ml of 30%) was added and the solution stirred at room temperature for an additional 24 hr; whereupon, palladium on charcoal was added and the solution stirred an additional 2 hr. Filtration and evaporation gave 1.1 g of an oily product. The nmr spectrum of a portion of this crude product showed the presence of only *threo*-2,3-dihydroxy-4-methylpentanoic acid (**29**). There were no peaks present from the isomeric erythro acid **28** (see below for synthesis of **28** and **29**). When some of the synthetic *threo* acid was added to the nmr tube, intensification of the already present peaks resulted. Addition of a small amount of the *erythro* acid resulted in the clear detection of its characteristic peaks in the spectrum. Glc (3% OV-17 column at 136°) of the silanized ($(CH_3)_3SiCl$) product showed only a peak with the same retention time as the synthetic *threo* acid and a different retention time from the synthetic *erythro* isomer. Finally the *threo* acid obtained by ozonolysis was isolated by preparative tlc on Merck Kieselgel PF_{254} using a solvent system of ethanol-water-25% aqueous NH_4OH (10:15:100). Under these conditions the *erythro* isomer gave R_f 0.58, while the *threo* isomer showed a R_f 0.53 by detection with iodine or $KMnO_4$. In a similar manner *trans*-2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran (mp 43–44°; $J_{2,3}$ 4.2 Hz) on ozonolysis gave exclusively *erythro*-2,3-dihydroxy-4-methylpentanoic acid, identified and characterized as described above.

STEREOSPECIFIC SYNTHESES OF *erythro* AND *threo*-2,3-DIHYDROXY-4-METHYLPENTANOIC ACIDS.—1,3-Dibromo-4-methyl-2-pentanone (bp 95–97°/10 mm $n^{25}d = 1.5099$) was prepared by bromination of methylisobutyl ketone and then submitted to the Favorsky reaction to give, in 80% yield, *cis*-4-methyl-2-pentanoic acid (**31**) according to the previously described procedure (55). It had the following physical properties: mp 15.5–17.5°; $n^{25}d = 1.4420$; δ(CCl_4) 1.20 (3 H, d, J = 7 Hz), 1.28 (3H, d, J = 7 Hz), 3.78 (1 H, m), 5.78 (1 H, d, J = 11.5 Hz), 6.22 (1 H, dd, J = 11.5, 9.5 Hz), 11.91 (1 H). *Cis*-4-methyl-2-pentanoic acid (**31**) was stereospecifically *cis* hydroxylated as follows. To 4.45 g of the *cis* acid in a solution of 150 ml of water, 100 ml of dioxane and 5 ml of a 1% solution of OSO_4 in an ice bath, was added 3 g of $AgClO_3$ in ¼ g portions over a period of 3 days, in the dark. The silver chloride precipitate was removed by filtration, and it was washed with dilute HCl. Hydrogen sulfide was bubbled through the filtrate for 0.5 hr, then the solution was neutralized with a sodium hydroxide solution. Evaporation of the solution gave a heavy syrup which was crystallized from ethyl acetate to give *erythro*-2,3-dihydroxy-4-methylpentanoic acid (**29**): mp 127°; Calc. for $C_6H_{12}O_4$: C, 48.64; H, 8.16. Found: C, 48.68; H, 8.00; νKBr 3250, 1680, 1390, 1355, 1290, 1230, 1130, 1095, 1020, 920, 840, 760 cm^{-1}; δ(D_2O): 0.94 (6 H, d, J = 6.75 Hz), 1.92 (1 H, m), 3.53 (1 H, dd, J = 6, 5.5 Hz), 4.29 (1 H, d, J = 5.5 Hz).

Threo-2,3-Dihydroxy-4-methylpentanoic acid (**28**) was prepared as follows. Malonic acid (83.2 g) was dissolved in a solution of 300 ml of pyridine and 4 ml of piperidine, then 57.6 g of isobutyraldehyde was added and the solution was refluxed for 24 hr. Removal of the water and pyridine at atmospheric pressure gave a crude product which was distilled to give 47.5 g (52%) of *trans*-4-methylpentanoic acid (**30**), bp 54–55°/0.15 mm, rpt. bp 100°/6 min (56); n_d 1.4481, rpt n_d 1.4487 (56); δ($CDCl_3$): 1.06 (6 H, d, J = 7 Hz), 2.45 (1 H, m), 5.74 (1 H, dd, J = 15.5, 1.5 Hz), 7.00 (1 H, dd, J = 15.5, 7 Hz), 12.45 (1 H). *Cis* hydroxylation of *trans*-4-methylpentanoic acid (4.4 g), as described above, gave 3.1 g (55%) of *threo*-2,3-dihydroxy-4-methylpentanoic acid (**28**): mp 111–112° (from ethyl acetate); Calc. for $C_6H_{12}O_4$: C, 48.64; H, 8.16. Found: C, 48.80; H, 8.05; νKBr 3250-3400, 1680, 1370, 1275, 1240, 1130, 1080, 1050, 1030, 915, 840, 785 cm^{-1}; δ(D_2O): 0.98 (6 H, d, J = 6.5 Hz), 1.83 (1 H, m), 3.54 (1 H, dd, J = 9.2 Hz), 4.42 (1 H, d, J = 2 Hz).

SYNTHESES OF *cis* AND *trans*-2-METHYL, 2-ETHYL, 2-n-PROPYL-3-HYDROXY-5-BROMO-2,3-DIHYDROBENZOFURAN.—Following the procedure previously described for the synthesis of 2'-hydroxy-2,5'-dibromo-3-methylbutyrophenone (**37**), 2'-hydroxy-2,5'-dibromopropiophenone 2'-hydroxy-2,5'-dibromobutyrophenone and 2'-hydroxy-2,5'-dibromovalerophenone were prepared.

2'-Hydroxy-2,5'dibromopropiophenone.—It exhibited the following physical properties: mp 98–99° (from EtOH); Calc. for $C_9H_8O_2Br_2$: C, 35.10; H, 2.62; Br, 51.89. Found: C, 35.14; H, 2.79; Br, 52.00; δ(CCl_4): 1.87 (3 H, d, J = 6.5 Hz), 5.16 (1 H, q, 5.16 Hz), 6.85 (1 H, d, J = 9 Hz), 7.49 (1 H, dd, J = 9.2 Hz), 7.82 (1 H, d, J = 2 Hz).

2'-Hydroxy-2,5'dibromobutyrophenone.—It exhibited the following properties: mp 56–57° (from EtOH); Calc. for $C_{10}H_{10}O_2Br_2$: C, 37.30; H, 3.13; Br, 49.63. Found: C, 36.91; H, 2.94; Br, 49.20; δ(CCl_4): 1.10 (3 H, t, J = 7.5 Hz), 2.12 (2 H, m), 5.92 (1 H, t, J = 7 Hz), 6.84 (1 H, d, J = 9 Hz), 7.48 (1 H, dd, J = 9.2 Hz), 7.79 (1 H, d, J = 2 Hz).

2'Hydroxy-,2'5dibromovalerophenone.—The following properties were observed: mp 55–56° (from EtOH); Calc. for $C_{11}H_{12}O_2Br_2$: C, 39.31; H, 3.60; Br, 47.56. Found: C, 38.84; H, 3.87; Br, 46.94; δ(CCl_4): 1.02 (3 H, t, J = 7.5 Hz), 1.50 (2 H, m), 2.08 (2 H, m), 5.98 (1 H, t, J = 7 Hz), 6.85 (1 H, d, J = 9 Hz), 7.48 (1 H, dd, J = 9.2 Hz), 7.78 (1 H, d, J = 2 Hz).

Following the proceedures previously described (37) for the preparation of *cis* and *trans*-2-isopropyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran, respectively, *cis* and *trans* 2-methyl, 2-ethyl and 2-n-propyl-3-hydroxy-2,3-dihydrobenzofurans were prepared. The compounds were only characterized by their ^1H nmr spectra, which are given below.

trans-2-Methyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran (**23**).—δ(CCl_4, D_2O): 1.24 (3 H, d, J = 6.5 Hz), 4.40 (1 H, m), 4.59 (1 H, d, J = 3.5 Hz), 6.57 (1 H, d, J = 8.5 Hz), 7.21 (1H, dd, J = 8.5, 2 Hz), 7.30 (1 H, d, J = 2 Hz).

cis-2-Methyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran (**22**).—δ(CCl_4, D_2O): 1.35 (3 H, d, J = 6.5 Hz), 4.43 (1 H, m), 4.68 (1 H, d, J = 5.5 Hz), 6.55 (1 H, d, J = 8.5 Hz), 7.19 (1 H, dd, J = 8.5, 2 Hz), 7.30 (1 H, d, J = 2 Hz).

trans-2-Ethyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran (**25**).—δ(CCl_4, D_2O): 1.05 (3 H, t, J = 7 Hz), 1.57 (2 H, m), 4.29 (1 H, m), 4.80 (1 H, d, J = 3.5 Hz); 6.59 (1 H, d, J = 9 Hz), 7.22 (1 H, dd, J = 9.2 Hz), 7.35 (1 H, d, J = 2 Hz).

cis-2-Ethyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran (**24**).—δ(CCl_4, D_2O): 1.08 (3 H, t, J = 7 Hz), 1.5–1.8 (2 H, m), 4.20 (1 H, m), 4.76 (1 H, d, J = 6 Hz), 6.56 (1 H, d, J = 9 Hz), 7.20 (1 H, dd, J = 9,2 Hz), 7.32 (1 H, d, J = 2 Hz).

trans-2-Propyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran (**27**).—δ(CCl_4, D_2O): 1.12 (3 H, t, J = 7 Hz), 1.51 (4 H, m), 4.32 (1 H, m), 4.74 (1 H, d, J = 3.7 Hz), 6.58 (1 H, d, J = 8.5 Hz), 7.22 (1 H, dd, J = 8.5, 2 Hz), 7.32 (1 H, d, J = 2 Hz).

cis-2-Propyl-3-hydroxy-5-bromo-2,3-dihydrobenzofuran (**26**).—δ(CCl_4, D_2O): 1.13 (3 H, t, J = 7 Hz), 1.51 (4 H, m), 4.31 (1 H, m), 4.82 (1 H, d, J = 5.5 Hz), 6.58 (1 H, d, J = 8.5 Hz), 7.23 (1 H, dd, J = 8.5, 2 Hz), 7.32 (1 H, d, J = 2 Hz).

ACKNOWLEDGMENTS

We express our sincere appreciation to the National Cancer Institute, NIH, for support of this work (CA-18819). We also thank Dr. C. Dickinson, Dr. M. Gordon, and Ms. S. Bonetti for performing various individual experiments related to this work. The FOFC tables have been deposited with the Editor.

Received 16 October 1978.

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Reprinted from JOURNAL OF NATURAL PRODUCTS, Vol. 42, No. 1, Jan-Feb, 1979

THE LOWER TERPENOIDS OF *ISOCOMA WRIGHTII*

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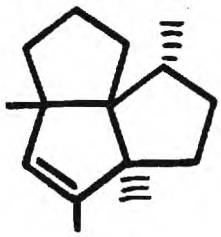
ABSTRACT.—The toxic plant "rayless goldenrod" (*Isocoma Wrightii≡Haplopappus heterophyllus*) has been shown to contain, in its volatile oil, three novel sesquiterpenes, isocomene (**1**), modhelphene (**2**), and the nordrimane sesquiterpene, 1,2,3,4-tetrahydro-1,1,5,6-tetramethylnaphthalene (**3**), in addition to β-caryophyllene, caryophyllene oxide and the monoterpenes limonene, borneol, bornyl acetate and carvone. The 1,1,5,8-tetramethyl (**4**) and 1,1,5,7-tetramethyl (**6**) isomers of **3** were synthesized and their nmr spectra compared.

"Milk sickness" has been described as the leading cause of death and disability in many parts of the midwest and upper South during the entire 19th Century (1). It was early established that the disease in animals ("trembles") was due to consumption of the plants white snakeroot (*Eupatorium urticaefolium≡E. rugosum*) east of the Mississippi and rayless goldenrod (*Haplopappus heterophyllus≡Isocoma wrightii*[1]) west of the Mississippi, and the toxic substance was passed on to humans ("milksickness") via the milk of the affected animal (1, 2). However, the toxin responsible for this disease has never been conclusively identified in spite of the numerous statements, even in recent times, in the literature reporting the toxin to be an unsaturated alcohol, "tremetol" ($C_{16}H_{22}O_3$) (1–4), first reported by Couch in the late 1920's (5–7). By the late 1930's, Dermer and his students (8–9) had already shown that "tremetol" was not a single pure substance but rather a complex mixture. Then, in the early 1960's Bonner (10) used Couch's procedure to reisolate "white snakeroot termetol" and further partitioned it into a sterol fraction and a ketone fraction, only the latter of which gave Couch's (5) characteristic sulfuric acid color test for "tremetol." This ketone fraction was shown to contain the benzofurans tremetone (2S-isopropenyl-5-acetyl-2,3-dihydrobenzofuran), hydroxytremetone and dehydrotremetone. At about the same time we began a reinvestigation of "rayless goldenrod tremetol" and identified toxol (2S, 3R-2-isopropenyl-3-hydroxy-5-acetyl-2,3-dihydrobenzofuran) and dehydrotremetone (11) and more recently tremetone, toxyl angelate, 2,5-diacetylbenzofuran and toxethol (2-isopropenyl-3-hydroxy-5-(1'-ethoxyethyl)-2,3-dihydrobenzofuran) (12). While none of the above-mentioned benzofurans has been implicated as the causative agent of "trembles" in higher animals, some of them have been shown to show biological activity.[2] The ethanolic plant extract of rayless goldenrod (*I. wrightii*) has been an unusually rich source of secondary plant metabolites of diverse structures. Thus, in addition to the above-mentioned benzofurans, the novel steroids 5α-androstane-3β, 16α, 17α-triol (13) and stigmasta-8(14), 22-dien-3β-ol (14) were isolated, and more recently we have identified stigmasta-5, 22-dien-3β-ol and stigmasta-8(14)-en-eβ-ol (12). The triterpenes friedelin and friedelan-3α-ol were obtained from the hexane extract (15), and now we have found squalene and phytol (12). Furthermore, the hexane extract has yielded a complex hydrocarbon fraction, and after chromatography we have isolated and identified nonacosane, hentri-

[1]*Isocoma wrightii* (Gray) Rydb. was formerly known as *Haplopappus* (*Aplopappus*) *heterophyllus*. See Cordell, D. S. and M. C. Johnston, 1970. Manual of Vascular Plants of Texas. Texas Research Foundation. Renner, Texas.

[2]A forthcoming publication entitled "The Benzofurans of *Isocoma wrightii*" will discuss this subject in detail.

acontane and tritriacontane (12). Among the fatty acids, stearic acid was isolated. Hexanoic, octanoic, lauric, myristic, palmitic and linoleic were identified by glc of their methyl esters (16). Finally, a family of fatty alcohols has been isolated but not yet completely characterized.[3]



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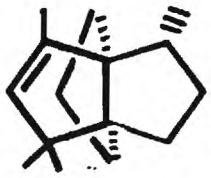

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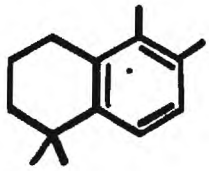

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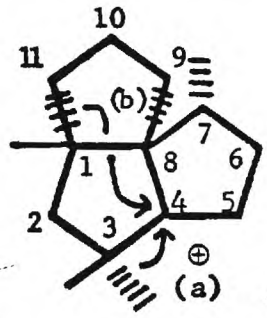
The sesquiterpenoid content of the volatile oil of *I. wrightii* has proven of particular interest from a structural point of view. Thus, in addition to the more common β-caryophyllene and its oxide we have isolated isocomene (**1**), a new sesquiterpene of novel skeletal type, the first sesquiterpenoid carbocyclic(3.3.3)-propellane, modhepene (**2**), and a novel nordrimane sesquiterpene, 1,2,3,4-tetrahydro-1,1,5,6-tetramethylnaphthalene (**3**). The structures of isocomene and modhephene were inferred from spectral analyses but only conclusively determined by single crystal X-ray analyses of the corresponding major diols derived by treatment of these tricycloalkenes with osmium tetroxide. The X-ray structures were recently presented in preliminary communications (17, 18), but full experimental details can be found in the EXPERIMENTAL of this paper.

The ^{1}H nmr spectra of isocomene (**1**) and modhephene (**2**) revealed that both substances possessed two quaternary methyl groups, a methyl group attached to a tertiary carbon, a methyl group attached to a trisubstituted double bond, and an olefinic hydrogen. In both of the nmr spectra of isocomene and modhephene, the two quaternary methyl groups appeared as singlets. In the case of isocomene this turned out to be misleading as will be evident. The ^{15}C nmr spectra of both isocomene and modhedphene verified that each contained a trisubstituted double bond and, in addition, revealed that each structure possessed three quaternary carbon atoms. The mass spectra of the two unknowns confirmed the molecular weights and elemental compositions deduced from the elemental analyses. The base peak in isocomene (*m/e* 189) seemed to correspond to the loss of propylene ($M^+-C_3H_6$), but in modhephene (*m/e* 162) it appeared to arise simply from the loss of a methyl group (M^+-CH_3). Thus, while the similarities in the ^{1}H nmr spectra suggested the two substances were skeletally related, the great differences in the ^{15}C nmr and mass spectra showed that isocomene and modhephene possessed different skeleta. Both substances were converted into major diols by treatment with osmium tetroxide in pyridine. The ^{1}H nmr spectrum of the major idol obtained from isocomene clearly showed three methyl singlets in addition to a methyl doublet, thus revealing that none of the quaternary carbons in isocomene bore a gem dimethyl group. Whereas the ^{1}H nmr spectrum of the major diol from modhephene showed a six proton methyl singlet analogous to that shown in modhephene, suggesting that one of the quaternary carbons in modhephene did bare a gem dimethyl group. We were only able to arrive at the unequivocal structures

[3]Unpublished work of B. Ekpo., Georgia Institute of Technology.

(1) and (2) for these two unknowns by single crystal X-ray analyses of the above mentioned diols (17, 18).

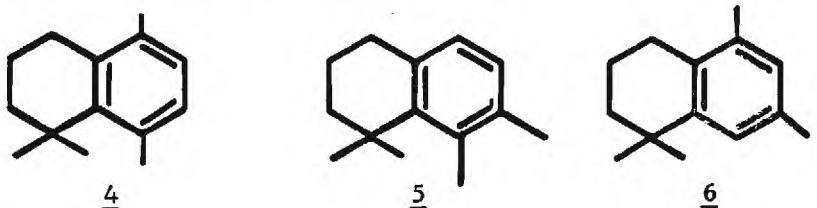
In the preparation of the *cis* diols of isocomene and modhephene, respectively, which were used for the above mentioned X-ray analyses, *cis* hydroxylation from one side predominated in each case. Thus, in the case of isocomene (1), the predominant isomer was formed by *cis* hydroxylation from the α side of (1) (*cis* to the two *cis* methyl groups), while in the case of modhephene (2), the predominant isomer was formed by α hydroxylation of (2) (*cis* to the unsubstituted bridge). A cursory examination of models of (1) and (2) fails to reveal any apparent steric or conformational preference for either face of the double bonds in these highly symmetrical tricycloalkenes. This remains an intriguing question. Another interesting observation is the difference in the magnitudes of the plain negative ord curves with the molecular rotation curve of isocomene being about ten times greater than that of modhephene. An examination of models of the two molecules clearly reveals that modhephene (2) is the more symmetrical of the two, and removal of the secondary methyl group would convert it into a non-chiral substance with a plane of symmetry running through the three carbon bridge bearing the gem dimethyl group and double bond. Since caryophyllene is by far the major sesquiterpenoid component of this plant, it is tempting to suggest it as a precursor to isocomene (1) and modhephene (2). It seems very likely that both these sesquiterpenes arise from the common intermediate carbonium ion indicated (scheme 1) via methyl migration (path a) to give isocomene (1) or migration of bond C(1)–C(11) to give modhephene (path b). The relative configurations of these two sesquiterpenes are consistent with this postulation.



Steam distillation of the hexane extract of the entire aboveground portion of the plant yielded a yellow essential oil which, upon distillation, gave a low boiling fraction containing (+) limonene, (−) borneol, (−) carvone, and bornyl acetate, which were isolated by chromatography on alumina. Chromatography of the higher boiling residue on silica gel yielded in some of the hexane eluents a homogeneous (glc) colorless liquid in 2% yield based on steam volatile oil. The analytical and spectral data suggested that this unknown possessed one of the structures **3–5**.

A synthesis of the more symmetrical isomer **4** was undertaken beginning with *p*-xylene by Friedel-Crafts acylation with succinic anhydride to give 4(2,5-dimethylphenyl)-4-oxo-butanoic acid, followed by Huang-Minlon reduction to 4(2,5-dimethylphenyl)butanoic acid, then esterification with diazomethane, followed by addition of the Grignard reagent methylmagnesium iodide to give 5(2,5-dimethylphenyl)-2-methyl-2-pentanol, and finally Friedel-Crafts alkylation

with polyphosphoric acid to give 1,1,5,8-tetramethyl-1,2,3,4-tetrahydronaphthalene (**4**). The *p*-xylene used in the synthesis contained a small amount of *m*-xylene, which reacted in a parallel series of reactions to give ultimately 1,1,5,7-tetramethyl-1,2,3,4-tetrahydronaphthalene (**6**). The two tetrahydronaphthalenes **4** and **6** were separated by chromatography on silica gel impregnated with silver nitrate, with **6** being eluted first. Tetralin **6** was identified by comparison of its ir and nmr spectra with those of an authentic sample prepared in a similar manner (19).[4] While the ir and mass spectra of synthetic **4** were similar to those of the unknown, the two differed in glc retention time, and the differences in their nmr spectra were particularly instructive. A comparison of the nmr spectra of **6** with that of **4** and the unknown suggested that the correct structure of the unknown was, in fact, **3** and not **5** because in both the unknown and in **6** the gem dimethyl group and the two aromatic methyl groups had almost identical chemical shifts respectively. Whereas, in **4** both the gem dimethyl group and the aromatic methyl group at C-8 showed rather considerable deshielding, as would be expected if the correct structure of the unknown were **5**.



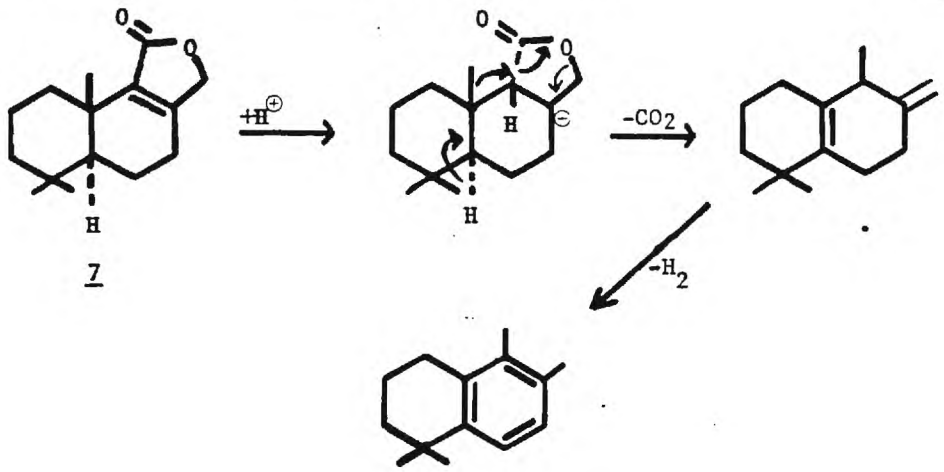
4 **5** **6**

A search of the literature revealed that 1,2,3,4-tetrahydro-1,1,5,6-tetramethylnaphthalene (**3**) had recently been reported as a rearranged degradation product of the sesquiterpene avarol formed on dehydrogenation with 10% Pd-C at 270° (20). Indeed, the reported spectral properties and a copy of the nmr spectrum verified that our unknown was identical to the above degradation product.[5] An examination of the carbon skeleton of 1,2,3,4-tetrahydro-1,1,5,6-tetramethylnapthalene (**3**) indicates that it is a nordrimane sesquiterpene with the bridgehead methyl group missing. Indeed, a mechanistically feasible pathway for its biogenesis can be visualized from the known sesquiterpene isodremenin (**7**) as outlined. The extremely mild isolation procedure used involving hexane extraction, steam distillation, fractional distillation, and finally chromatography on alumina make it highly unlikely that **3** is an artefact. Indeed, we know of no precursors, including isodremenin (**7**) which would lead to **3** under these conditions.

Saponification of the methanolic plant extract gave an ether soluble dark red oil (1% based on dried plant) which on steam distillation gave an essential oil which upon further fractional distillation gave (−) carvone, (−) borneol and (−) caryophyllene. Chromatography of the fraction bp 75–95°/0.05 mm on alumina (act III) gave (−) caryophyllene oxide. Each of these terpenes was identical, within experimental error, by ir, nmr and $[\alpha]_D$ with authentic samples. When the methanolic plant extract was diluted with an equal volume of water then extracted with pentane, (+) limonene was obtained, identical by ir, nmr and $[\alpha]_D$ with an authentic sample.

[4]We are grateful to Professor Nasipuri, Department of Chemistry, Indian Institute of technology, Kharagur, India for the ir and nmr spectra of **6**.

[5]We thank: Professor Minale, Consiglio Nazionale Delle Richerche, Laboratorio per la Chimica di Molecole Interesse Biologio, Napoli, Italy, for a copy of the nmr spectrum of **3**.



7

EXPERIMENTAL[6]

ISOLATION OF 1,2,3,4-TETRAHYDRO-1,1,5,6-TETRAMETHYLNAPHTHALENE (3).—The aboveground parts of rayless goldenrod (*I. Wrightii*) were collected in the vicinity of Artesia, New Mexico, in October 1968, air dried, ground, and stored in sealed bags until extracted in 1974. Ten kilograms of the plant material was extracted with 16 liters of hexane in a Soxhlet for 2 days. Removal of the hexane with a rotary evaporator gave 170 g of extract. Steam distillation and ether extraction of 350 g of this extract gave 6.8 g of yellow oil after drying and removal of the ether with a rotary evaporator. Distillation of this oil yielded a lower boiling fraction (b.p. 26–36°C/0.15 mm) from which (+) limonene, (−) borneol, (−) carvone, and bornyl acetate were isolated by chromatography on Brochman alumina (act. II) and a residue (2.1 g) which was chromatographed on 72 g of silica gel. Among the hexane eluents, there was obtained a homogeneous (glc) oil in about 2% yield based on steam volatile oil. Bp 75–80°/0.2 mm (air bath); ν max (film) 800 cm^{-1}; λ max (MeOH) 269, 277 nm; nmr (CCl_4) δ 1.26 (6H, s), 2.10 (3H, 3), 2.24 (3H, s), 2.66 (2H, t, J=6 Hz), 6.92 (2H, q, J=8 Hz); mass spectrum m/e 188 (M^+, 18%), 173 M^+-CH_3, 100%). Anal. Calcd. for $C_{14}H_{20}$: C, 89.30; H, 10.70. Found: C, 89.37; H, 10.61.

SYNTHESIS OF 1,1,5,8-TETRAMETHYL-1,2,3,4-TETRAHYDRONAPHTHALENE (4) AND 1,1,5,7-TETRAMETHYL-1,2,3,4-TETRAHYDRONAPHTHALENE (6).—To an ice-cooled solution prepared by adding 14.3 g of succinic anhydride to 130 ml of commercial *p*-xylene was added 50 g of anhydrous $AlCl_3$ with stirring. After warming to room temperature, the solution was heated on the steam bath for 20 min; then 75 ml of H_2O was added dropwise while the reaction mixture was cooling in an ice bath. Excess xylene was removed by steam distillation; and, on cooling in an ice bath, the crude 4(2,5-dimethylphenyl)-4-oxobutanoic acid separated as an oil on top of the solution and soon solidfied. The solid was removed by filtration, washed successively with ice cold 2N HCl and ice water, then dissolved in 15% Na_2CO_3. The solution was filtered and decolorized with charcoal. Acidification with conc. HCl precipated the acid, which was collected by filtration, dried, and recrystallized from EtOH-H_2O to give mp 66–68°, yield 46% (lit. ref. 21, mp 86°). ν max ($CHCl_3$) 3500–2500 br, 1701, 1680 cm^{-1}; nmr (CCl_4) δ 2.36 (3H, s), 2.43 (3H, s), 2.73 (2H, t, J=6 Hz), 3.13 (2H, t, J=6 Hz). The low mp and complex aromatic region in the nmr spectrum was due to the presence of a small amount of 4(2,4-dimethylphenyl)-4-oxobutanoic acid arising from m-xylene contaminant in the p-xylene. It was more convenient to separate isomers at a later stage.

To 10 g of KOH dissolved in 57 ml of diethylene glycol at 80–100° was added 10.4 g of the above ketoacid mixture and 7.3 ml of 85% hydrazine hydrate. The solution was refluxed for 1 hr. Then the low boiling materials were distilled out until the pot temperature reached 205°, when the solution was again allowed to reflux for 1 hr. After cooling, the solution was poured into H_2O and acidified with conc. HCl, whereupon crude 4(2,5-dimethylphenyl)butanoic acid

[6]MP's were taken on a Kofler hot stage and are uncorrected. Ir spectra were recorded with a Perkin Elmer 237 B spectrophotometer. ^1H nmr spectra were obtained with a Varian A-60D or T60 spectrometer with Me_4Si as an internal standard (δ 0); ^{13}C nmr spectra were run on a JOEL-PFT-100 FT spectrometer. Mass spectra were run on a Hitachi RMV-7 spectrometer; gas chromatography was done with a F&M Biomedical Gas Chromatograph, model 402; and ord spectra were recorded using a Jasco ORD/UV-5 instrument.

oiled out and then solidfied. After purification as described above, the product was recrystallized from pentane to give mp 57–59°, yield 97% (lit. ref. 22, m.p. 70–72°); ν max ($CHCl_3$) 3500–2500 br, 1705 cm^{-1}. The acid mixture (8.4 g) was treated with excess ethereal diazomethane and allowed to stand at room temperature overnight. Water was added, the ether layer was removed and washed successively with water, ice cold 5% NaOH, then saturated brine and finally dried over $MgSO_4$ to give the crude methyl ester mixture (ν max (film) 1740 cm^{-1}) in 48% yield. Gas chromatography on an OV-17 column at 140° clearly showed the presence of the two esters methyl-4(2,5-dimethylphenyl)butanoate and methyl-4(2,4-dimethylphenyl)butanoate in a ratio of 2:1. A 60 MHz nmr spectrum did not distinguish between the two isomers: δ 2.33 (6H, s), 3.70 (3H, s).

A solution of 4.6 g of the above ester mixture dissolved in 44 ml of anhyd. ether was dropped slowly into an ethereal solution containing a large excess of methylmagnesium iodide prepared in situ. The solution was refluxed for one hr. then cooled in an ice bath and hydrolyzed by the slow addition of 40 ml of 20% ammonium chloride. The usual workup gave a 93% yield of the mixture of alcohols 5-(2,5-dimethylphenyl)-2-methyl-2-pentanol and 5(2,4-dimethylphenyl)-2-methyl-2-pentanol in a ratio of 2:1 respectively (glc, same column as above). Bp 103–105°/0.35 mm; ν max (film) 3200–3500 br; δ 1.20 (6H, s), 2.30 (6H, s), isomers not distinguishable; *m/e* 132 (100%).

A solution prepared by dissolving 1 g of the above alcohol mixture in 17 g of polyphosphoric acid was heated at 160° for 3 hrs. It was then cooled, poured onto crushed ice in water, and the latter was extracted with hexane. The combined hexane extracts were washed successively with 5% NaOH and saturated brine and finally dried over $MgSO_4$. Removal of the solvent gave 0.63 g of oil, bp 68–69°/0.15 mm, and nmr indicated that this oil was a mixture of **4** and **6** in ° ratio of 2:1, and, therefore, it was chromatographed on silica gel impregnated with 20% $AgNO_3$. Elution with hexane-CH_2Cl_2 (85:15) gave first the minor component **6** and then the major component **4**. 1,1,5,7-tetramethyl-1,2,3,4-tetrahydronaphthalene (**6**) was identical by ir and nmr spectra with those of an authentic sample (19). ν max (film) 855 cm^{-1}; δ (CCl_4): 1.25 (6H, s), 2.13 (3H, s), 2.23 (3H, s), 6.70 (1H, s), 6.92 (1H, s). 1,1,5,8-Tetramethyl-1,2,3,4-tetrahydronaphthalene (**4**) was isolated as a colorless oil. Bp ~80°/0.2 mm (air bath); ν max (film) 807 cm^{-1}; δ (CCl_4) 1.38 (6H, s), 2.10 (3H, s), 2.43 (3H, s), 6.73 (2H, s); mass spectrum *m/e* 188 (M^+, 48%), 173 (100%). Anal Calcd. for $C_{14}H_{20}$: C, 89.30; H, 10.70. Found: C, 89.18; H, 10.81.

ISOLATION OF ISOCOMENE (**1**), MODHEPHENE (**2**), CARYOPHYLENE, CARYOPHYLENE OXIDE AND MONOTERPENES.—The aboveground parts of rayless goldenrod (10 kg) were extracted with 10 liters of methanol to give 300 g of extract, which was saponified with 5% methanolic KOH and extracted with ether to give 30 g of red oil ("tremetol"). Steam distillation of the latter gave in 11% yield the essential oil. When some of the original methanol extract was diluted one to one with water, it became cloudy. On extraction with ligroin, drying, and evaporation, a colorless oil was obtained which by glc showed one major (60%) component. This component was isolated by fractional distillation and identified by its physical and spectral properties as (+) limonene.

The essential oil from above was distilled to give fractions of the following boiling points at 0.05 mm: A, 40–55°; B, 55–65°; C, 65–75°, and D, 75–95°. Refractionation of fraction A gave a fraction of bp 65–73°/1 mm which was chromatographed on alumina to yield (−) carvone in the benzene-chloroform (1:1) eluent, and (−) borneol in the chloroform eluent. The fraction of bp 75–80°/1 mm (from fraction A) gave β-caryophyllene, which was also isolated directly from "tremetol" by partition chromatography on florisil (stationary phase 95% methanol saturated with ligroin). Fraction C was chromatographed on silica gel impregnated with silver nitrate (20%) to give β-caryophyllene, isocomene (**1**) (17), and modhephene (**2**) (18) in the pentane-methylene chloride eluents.

Pentane-methylene chloride (95:5) eluted first isocomene, then modhephene, and finally caryophyllene. Of this mixture of sesquiterpenes, caryophyllene comprised about 64%; isocomene, 26%; and modhephene, 10%. All were distinguishable by glc on a 6′ x ¼″ 5% SE 30 column. Isocomene (**1**) was obtained as a colorless oil (single peak by glc) which crystallized after standing at room temperature for several months. Bp 65–70°/0.35 mm (air bath); mp 60–62°; ν max (CCl_4) 3020, 1670 and 840 cm^{-1}; ^1H nmr δ(CCl_4) 0.87 (3H, d, *J* 7 Hz), 1.02 (6H, s), 1.67 (3H, d, *J* 1.5 Hz), 4.83 (1H, m); ^{13}C nmr 142.1(s), 132.1(d), 63.6(s), 59.7(s), 56.4(s), 42.5(t), 39.8(d), 37.2(t), 33.6(t), 31.9 (t), 24.0 (t), 23.7(q), 23.1(q), 17.3(q), 13.0(q) ppm; *m/e* 204(M^+, 15%), 189(19%), 162 (100%), 147(42%), 119(35%); ord (C, 1.18; $CHCl_3$): $[\phi]_{600}$ −129.7, $[\phi]_{589}$ −138.3, $[\phi]_{550}$ −155.7, $[\phi]_{500}$ −172.8, $[\phi]_{450}$ −259.3, $[\phi]_{400}$ −363.1, $[\phi]_{350}$ −535.9, $[\phi]_{300}$ −881.7, $[\phi]_{260}$ −1694.2°. Anal. Calcd. for $C_{15}H_{24}$: C, 88.16; H, 11.84, Found: C, 88.11; H, 11.88. On treatment of isocomene with osmium tetroxide in pyridine (for conditions, see modhelphene diol preparation), a mixture of cis diols was obtained in an approximate ratio of 3:2 as determined by ^1H nmr (isomers were not distinguishable on several glc columns). The major isomer was obtained pure by chromatography on silica gel and crystallization from pentane-ether. Mp 134–136°; ν max ($CDCl_3$) 3540, 3590 cm^{-1}; ^1H nmr δ ($CDCl_3$) 0.91 (3H, d, *J* 6.51 Hz), 0.94 (3H, s), 1.03 (3H, s), 1.15 (3H, s), 3.50 (1H, d, *J* 8 Hz): *m/e* 238 (M^+, 2%), 220 (M^+–H_2O, 29%), 134(30%), 122(90%), 109(100%); ord (C, 1.18; $CHCl_3$) $[\phi]_{589}$ −46.6°. Anal. Calcd. for $C_{15}H_{26}O_2$: C, 75.58; H, 10.99. Found: C, 75.54; H, 11.04. This diol was used for a single crystal X-ray analysis (17).

Modhephene (**2**) was isolated as a colorless oil from the chromatography and showed a single peak by glc. Bp 65–70°/0.25 mm (air bath); ν max (CCl_4) 3010, 1650, 1380, 840 cm^{-1}; ^1H nmr δ (CCl_4) 0.97 (6H, s), 0.99 (3H, d, J 5.5 Hz), 1.58 (3H, d, J 1.5 Hz), 4.80 (1H, m); ^{13}C nmr 140.2(s), 134.8(d), 71.9 (s), 65.9(s), 45.7(s), 43.8, 38.7, 35.7, 34.2, 29.9, 29.2, 27.1, 26.4, 15.7, 13.7 ppm; m/e 204 (M^+, 19%), 189 (100%), 161(29%), 149(36%), 147(30%), 133(26%), 119(32%); ord (C, 1.50; $CHCl_3$): $[\phi]_{600}$ −8.2°, $[\phi]_{589}$ −8.6°, $[\phi]_{550}$ −13.7°, $[\phi]_{500}$ −16.3°, $[\phi]_{450}$ −19.0°, $[\phi]_{400}$ −30.0°, $[\phi]_{350}$ −46.0°, $[\phi]_{300}$ −84.3°, $[\phi]_{260}$ −182.2°. Anal. Calcd. for $C_{15}H_{24}$: C, 88.16; H, 11.84. Found: C, 88.01; H, 11.89.

Modhephene (61 mg) was added to a solution containing 250 mg osmium tetroxide in 5 ml of dry pyridine. After stirring in the dark at room temperature for 10 days, a solution of 1 g $NaHSO_3$ dissolved in 10 ml water was added. After stirring for an additional 0.5 hr, 10 ml of half saturated brine solution was added, and the entire stirred solution was finally extracted with $CHCl_3$. The $CHCl_3$ extract was washed with 3M aq HCl then saturated brine solution and finally dried over $MgSO_4$. Evaporation under reduced pressure gave 66 mg of a light brown oil which solidified on standing at room temperature overnight. Glc analysid (5% SE-30 column) showed one major component and three minor ones. The minor component of shortest retention time was unreacted modhephene; whereas the minor component of retention time closest to the major component is presumably the isomeric cis diol. The apparent ratio of the major and minor diols is 4:1. Chromatography of the crude product on Merck acid washed alumina, act III, gave 41 mg of the diol mixture in the hexane-benzene (1:1) eluent as a crystalline material. Fractional crystallization by vapor diffusion with ether as the solvent and pentane as the external liquid gave colorless prisms of the major diol with properties listed below. A small piece of one of these crystals was cut off and used for the X-ray analysis (18). Mp 145–145.5°; ν max (KBr) 3500, 3375, 1070 cm^{-1}; ^1H nmr δ ($CDCl_3$) 0.98 (3H, d, J 6 Hz), 1.00 (6H, s), 1.25 (3H, s), 3.38 (1H, br d, J 6 Hz): m/e 238 (M^+, 8%), 220 (M^+-H_2O, 40%), 192(73%), 164(90%), 136(100%), 124(77%), 110(75%), 96(77%). Anal. Calcd. for $C_{15}H_{26}O$: C, 75.58; H, 10.99. Found: C, 75.54; H, 10.98. Fraction D was chromatographed on neutral alumina (act III) to give caryophyllene oxide in the benzene-hexane (1:3). All of the known terpenes isolated from the essential oil were identified by comparison of their physical and spectral properties with those of authentic samples.

ACKNOWLEDGMENT

We express our sincere appreciation to the National Cancer Institute, NIH, for support of this research. (R01–CA–18819).

Received 23 June 1978.

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Modhephene: a Sesquiterpenoid Carbocyclic [3.3.3]Propellane. X-Ray Crystal Structure of the Corresponding Diol

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Reprinted from

Journal of The Chemical Society

Chemical Communications

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The Chemical Society, Burlington House, London W1V 0BN

Modhephene: a Sesquiterpenoid Carbocyclic [3.3.3]Propellane. *X*-Ray Crystal Structure of the Corresponding Diol

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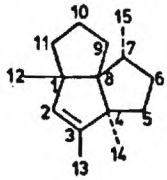
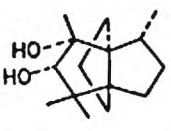
Summary Modhephene (**2**), a new sesquiterpene representing the first naturally occurring carbocyclic [3.3.3]propellane, has been isolated from the toxic plant *Isocoma Wrightii;* its structure was confirmed by *X*-ray analysis of the corresponding diol (**3**).

RAYLESS GOLDENROD (*Isocoma Wrightii*) has been a rich source of novel compounds such as benzofurans,[1] steroids,[2] and the unusual sesquiterpene isocomene (**1**).[3] We now report the isolation of another unexpected compound, namely, the first natural sesquiterpenoid carbocyclic [3.3.3]-propellane, which we have given the trivial name modhephene (**2**).

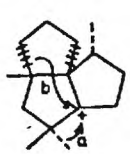


(1)

(2)



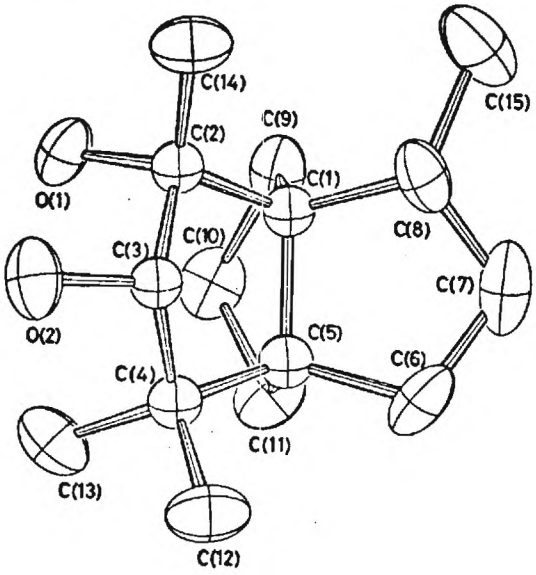
(3)



(4)

Steam distillation of the saponified methanolic extract of the dried leaves and stems provided a yellow oil which was fractionally distilled. The fraction of b.p. 65—75 °C at 0·05 mmHg was chromatographed on silica gel impregnated with silver nitrate (20%) to give, in the pentane–methylene chloride (95:5) eluent, first isocomene (**1**) and then modhephene (**2**) [ratio of (**1**) to (**2**) 2:1] as a colourless oil, b.p. 65—70 °C at 0·25 mmHg (bath), *m/e* 204 (M^+, 19%), 189(100%), 161(29), 149(36), 147(30), 133(26), and 119(32); ν_{max} (CCl_4) 3010, 1650, 1380, and 840 cm^{-1}; ^1H n.m.r.: δ 0·99 (3 H, d, *J* 5·5 Hz), 0·97 (6 H, s), 1·58 (3 H, d, *J* 1·5 Hz), and 4·80 (1 H, m); ^{13}C n.m.r.: δ 45·7, 65·9, 71·9 (s, quaternary C's), 134·8 (d, olefinic C with single H), and 140·2 (s, olefinic C with no H) p.p.m.

On treatment with osmium tetroxide in pyridine, (**2**) gave a 4:1 mixture of *cis* diols, from which the major diol (**3**) was separated by chromatography on alumina (activity III), m.p. 145—145·5 °C; *m/e* 238 (M^+, 8%), 220 ($M^+ - H_2O$, 40%), 192(73), 164(90), 136(100), 124(77), 110(72), and 96(76); ν_{max} (KBr) 3500, 3375, and 1070 cm^{-1}; δ 0·98 (3 H, d, *J* 6 Hz), 1·00 (6 H, s), 1·25 (3 H, s), and 3·38 (1 H, br d, *J* 6 Hz). The structure of the diol (**3**) was established by a single crystal *X*-ray analysis (Figure). It crystallized from ether–pentane (vapour diffusion) in the orthorhombic space group $P2_12_12_1$ with $a = 8·456(3)$, $b = 9·877(4)$, $c = 16·467(6)$, $Z = 4$. The intensity data were measured with a Syntex $P2_1$ four-circle diffractometer, equipped with a graphite monochromator, using the θ–2θ scan technique. The structure was solved using direct methods. An *E*-map generated by MULTAN contained peaks corresponding to all non-hydrogen atoms in the molecule. Two cycles of least squares refinement gave $R = 0·16$. Hydrogens were then located from a



combination of difference Fourier peaks and calculated positions. The final refinement gave $R = 0{\cdot}055$ and $R' = 0{\cdot}058$ for 1168 reflections with $I > 3\ \sigma(I)$† (see Figure). The temperature factors of the oxygens and C(7)—C(15) were varied anisotropically while those of the remaining atoms were isotropic and the hydrogen temperature factors were fixed at 5·0. The hydrogen co-ordinates were not varied.

Since caryophyllene is the major sesquiterpenoid component obtained from the essential oil of *I. Wrightii*, it is tempting to suggest it as a precursor to isocomene (**1**) and modhephene (**2**) and indeed it is possible to write such a biogenesis. However, it does seem very likely that isocomene (**1**) and modhephene (**2**) arise from the common precursor (**4**) *via* methyl migration (path a) to give isocomene (**1**) or migration of bond C(1)–C(11) to give modhephene (**2**) (path b). The relative configurations of these two sesquiterpenes are consistent with this postulation.

We thank the National Cancer Institute for financial support.

(*Received*, 10*th January* 1978; *Com.* 023.)

† The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication. The structure factor table may be obtained as a Supplementary publication (SUP 22300; 7 pp.) from the British Library. For details of obtaining this material, see Notice to Authors No. 7, Index Issues of *J.C.S. Perkin I* or *II*.

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Isocomene: a Novel Sesquiterpene from *Isocoma Wrightii*. *X*-Ray Crystal Structure of the Corresponding Diol

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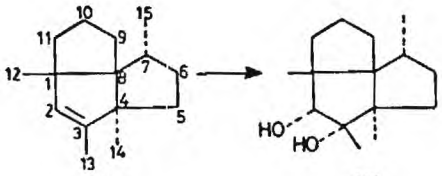
Summary Isocomene (**1**), a new sesquiterpene representing a novel skeletal type, has been isolated from the toxic plant *Isocoma Wrightii*; its structure was confirmed by *X*-ray analysis of its corresponding diol (**2**).

RAYLESS GOLDENROD (*Isocoma Wrightii*†) is a plant toxic to cattle and sheep[1] but the exact nature of the toxin remains unresolved. The plant has been shown to contain the bacteriostatic agent toxol[1,2] and related benzofurans,[3] and the novel steroids 5α-androstane-3β,16α,17α-triol[4] and

† Formerly known as *Haplopappus heterophyllus* (D. S. Correll and M. C. Johnston, 'Manual of Vascular Plants of Texas,' Texas Research Foundation, Renner, Texas, 1970).

stigmasta-8(14),22-dien-3β-ol.[5] Only recently have sesquiterpenes been found in *I. Wrightii*. Thus, Bohlmann and Zdero[6] reported the presence of three 8-oxo-β-cyperons in the roots and we have isolated caryophylene and caryophylene oxide from the stems and leaves.[7] We report here the isolation and structure of a new sesquiterpene of novel skeletal type from *I. Wrightii* and have given it the trivial name isocomene (**1**).



(1) (2)

Isocomene (**1**) was isolated from the dried stems and leaves by extraction with hexane or from the saponified methanol extract by steam distillation followed by fractional distillation. The fraction of b.p. 65—75 °C at 0·05 mmHg was shown by g.l.c. to be composed of *ca.* 90% caryophyllene and 10% (**1**). Chromatography on silica gel impregnated with silver nitrate (20%) gave (**1**)‡ in the hexane–methylene chloride (95 : 5) eluent as a colourless oil, b.p. 65—70 °C at 0·35 mmHg (bath), *m/e* 204 (M^+, 15%) 189 (19%), 162 (100%), 147 (42%), and 119 (35%); ν_{max} (CCl_4) 3020, 1670, and 840 cm^{-1}; ^1H n.m.r.: δ 0·87 (3H, d, J 7 Hz), 1·02 (6H, s), 1·67 (3H, d, J 1·5 Hz), and 4·83 (1H, m); ^{13}C n.m.r.: δ 56·4, 59·7, 63·6 (s, quaternary C's), 132·1 (d, olefinic C with single H), and 142·1 (s, olefinic C with no H) p.p.m.

Elemental analysis and ^1H and ^{13}C n.m.r. and mass spectra indicated that (**1**) was tricyclic, contained a trisubstituted double bond with an attached methyl group, two additional methyl groups attached at quaternary carbons, a methyl group at a tertiary carbon, and finally a quaternary carbon bearing no methyl groups. By analogy to known sesquiterpene skeleta[8] the six-proton n.m.r. singlet at δ 1·02 was assumed to arise from a *gem*-dimethyl group and only after the *X*-ray analysis, described below, was it clear that, in fact, this was not the case and (**1**) actually represented a previously unknown sesquiterpene skeleton.

On treatment with osmium tetroxide in pyridine, (**1**) gave a mixture of diols from which the isomer (**2**)‡ was separated by crystallization from pentane–ether and chromatography on silica gel, m.p. 134—136 °C; *m/e* 238 (M^+. 2%), 220 ($M^+ - H_2O$, 29%), 134 (30%), 122 (90%), and 109 (100%); ν_{max} ($CDCl_3$) 3540 and 3590 cm^{-1}; δ 0·91 (3H, d, J 6·5 Hz), 0·94 (3H, s), 1·03 (3H, s), 1·15 (3H, s), and 3·50 (1H, d, J 8 Hz). The structure of the diol (**2**) was established by a single crystal *X*-ray analysis (Figure). It crystallized from

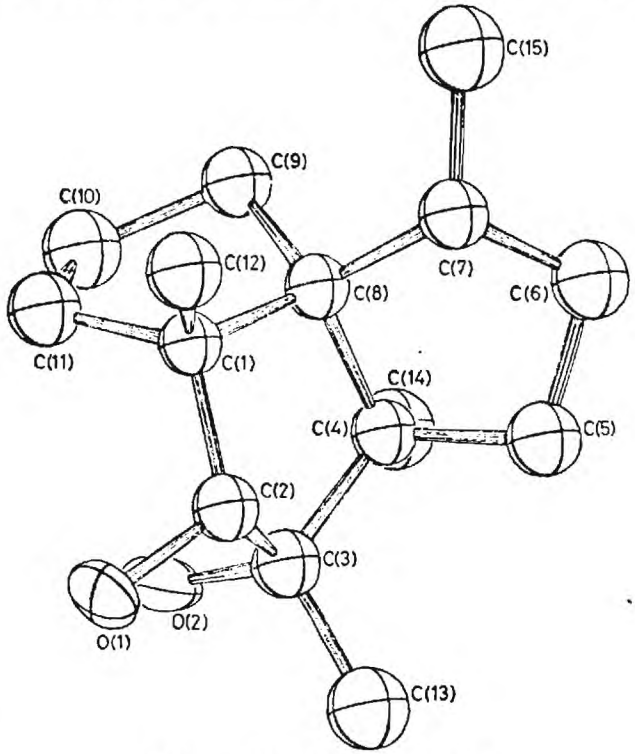


FIGURE. Structure of the diol (**2**).

pentane in the orthorhombic space group $P2_12_12_1$ with $a = 6·898(2)$, $b = 12·397(6)$, $c = 16·042(6)$ Å, $Z = 4$. The intensity data were measured with a Syntex P2, four-circle diffractometer, equipped with a graphite monochromator, using the θ-2θ scan technique. The structure, which was solved by direct methods, was refined by least-squares methods to convergence of $R = 0·069$ for 1046 reflections with $I > 3\sigma(I)$. Variables included a scale factor, co-ordinates of all carbon and oxygen atoms, anisotropic thermal parameters for oxygen and selected carbon atoms, and isotropic thermal parameters for the remaining carbon atoms. However, parameters were not varied for hydrogen atoms; fixed thermal parameters of 5·0 were used.§

We thank the National Cancer Institute for support.

(*Received, 24th January* 1977; *Com.* 058.)

‡ These compounds gave satisfactory elemental analyses.

§ The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Rd., Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication. The structure factor table may be obtained as a Supplementary publication (No. 22118, 6 pp.) from the British Library. For details of obtaining this material, see Notice to Authors No. 7, Index Issues of *J.C.S. Perkin I* or *II*.

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THE CO-OCCURRENCE OF DESMETHYLENCECALIN AND HYDROXYTREMETONE IN *EUPATORIUM RUGOSUM*

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(*Received* 1 *February* 1977)

Key Word Index—*Eupatorium rugosum*; Compositae; desmethylencecalin (7-hydroxy-2,2-dimethyl-6-acetyl-2H-chromene); hydroxytremetone (dehydroeuparin; 6-hydroxy-2-isopropenyl-5-acetylcumaranone).

Eupatorium rugosum (syn *E. urticaefolium*) was collected at Unicoi Gap, Appalachian Trail, Highway 75 north Georiga in September and October, 1976. Previous work on this plant includes the isolation and identification of tremetone, dehydrotremetone and hydroxytrometone [1]. The plant is responsible for 'milk sickness' in cattle and humans [1, 2]. Roots of freshly collected *E. rugosum*, in bloom, were macerated in 95% EtOH then continuously extracted with 95% EtOH to give 2% extract based on wet plant. Partition between 5% HCl and $CHCl_3$ yielded 16% of the extract in the organic layer, 43% of which was hexane soluble. GLC of the hexane soluble fraction showed five components (OV-17 column), the major component (~50%) being desmethylencecalin (**1**) and a minor component (~7%) having the same retention time alone and on admixture as authentic hydroxytremetone (**2**) on both OV-17 and SE-30 columns. Chromatography of the hexane soluble viscous oil on Si gel (230–400 mesh) and elution with hexane gave desmethylencecalin as orange crystals, mp 74–76°, rpt. [3] mp 77°, $\nu_{max}^{CCl_4}$ 3400–2500 (broad), 1645 cm^{-1}; δ (CCl_4) 1.39 (6H, *s*), 2.41 (3H, *s*), 4.50 (1H, *d*, J = 10 Hz), 6.21 (1H, *d*, J = 10 Hz), 6.21 (1H, *s*), 7.20 (1H, *s*), 12.44 (1H, *s*); MS: M^+ 218 (16%), 204 (100%), 202 (12%) 185 (10%). Identical with IR and ^{1}H NMR spectra of an authentic sample of desmethylencecalin [4].

Hydroxytremetone (**2**) was more readily isolated as follows. The EtOH extract (138 g) of the above ground portion of the green plant was steam-distilled and the aq. soln and insoluble portion were successively extracted with hexane, Et_2O and finally $CHCl_3$, then the aq. soln was filtered and evaporated to yield 91 g of residue. This residue was refluxed in 2N aq. EtOH–HCl, then extracted with Et_2O to give 15 g of residue which was refluxed in 10% ethanolic NaOH. Extraction of the latter solution with Et_2O (washing H_2O), drying and evaporation gave 0.7 g of residue which was chromatographed on Si gel (100–200 mesh) to give 190 mg of almost pure (**2**) in the hexane–EtOAc (4:1) eluent. Analytically pure (single peak by gas chromatography on SE-30 column) hydroxytremetone (**2**) was obtained by rechromatography on Si gel (100–200 mesh) and crystallization from hexane. Mp 63–65°, rpt. mp 70–71°, 69°; $[\alpha]_D^{25°}$ − 43° (*c*, 0.40, EtOH), rpt. $[\alpha]_D^{24°}$ − 50.7° (*c*, 0.74, EtOH): $\nu_{max}^{CCl_4}$ 1642: λ_{max} (EtOH) 208, 213, 232, 237, 280 and 335 nm; δ ($CDCl_3$) 1.75 (3H, *s*), 2.53 (3H, *s*), 2.95 (1H, *ddd*, J = 15, 9, 1), 3.29 (1H, *ddd*, J = 15, 9, 1), 4.93 (1H, *bs*), 5.07 (1H, *bs*), 5.26 (1H, *dd*, J = 9.9), 6.32 (1H, *s*), 7.48 (1H, *s*), 12.98 (1H, *s*); MS: Calc. for $C_{13}H_{14}O_3$: 218.0943, found, 218.0965; M^+ 218 (100), 203 (M^+-Me, 72%), 175 (M^+-MeCO, 42%). The UV, IR, ^{1}H-NMR and MS were identical, within experimental error, with the values reported in the literature and the IR and NMR spectra were identical, within experimental errors, to spectra of an authentic sample.

To our knowledge, the only prior report of the occurrence of desmethylencecalin has been in *Helianthella uniflora* where it co-occurs with hydroxytremetone [3]. Whereas hydroxytremetone has previously been reported in *E. rugosum* [1], its co-occurrence with desmethylencecalin is of particular phytochemical interest.

Acknowledgements—We express our sincere appreciation to the National Cancer Institute for support on this research (1 RO1-CA-18819-ol) and to Prof. F. Bohlmann, Institut für Organische Chemie der Technischen Universität Berlin for NMR and IR spectra.

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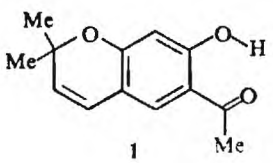

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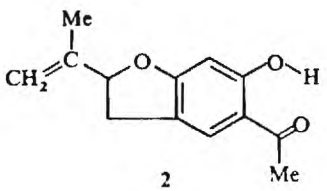

2

393(17), 219(30), 204(37), 203(100), 191(72), 190(56), 189(96), 187(36), 177(28), 175(33), 163(37). La réduction de (3) par le $NaBH_4$ dans le MeOH fournit le composée (2) (R_f, SM, IR, RMN, F). De même, l'oxydation de (2) par la méthode de Jones conduit à l'acétyl-3 moraldéhyde (3) (R_f, RMN, IR, SM, F).

Remerciements—Nous remercions MM les Professeurs F. Malaisse (UNAZA, Zaïre) pour l'identification du matériel végétal, Van Binst (VUB) pour les spectres SM en double focalisation et R. Huls (Univ. Liège) pour les spectres de CD, ORD et RMN du ^{13}C. Nous sommes reconnaissants à M. le Professeur J. Pécher de l'ULB qui a permis à l'un d'entre nous d'achever dans ses laboratoires la séparation des triterpénoïdes d'*Agauria salicifolia*.

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TRITERPENESE OF *ISOCOMA WRIGHTII*

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(*Received* 18 *April* 1977)

Key Word Index—*Isocoma Wrightii* (*Haplopappus heterophyllus*); Compositae; friedelin, friedelan-3α-ol.

Plant. Isocoma Wrightii (*Haplopappus heterophyllus*). Source, general area of Roswell, New Mexico. Previous work, isolation of benzofurans [1–4], steroids [5, 6], mono [7] and sesquiterpenes [7, 8] and fatty acids [7].

Present Work. The entire dried, above ground plant was ground and continuously extracted with hexane. Steam distillation of the hexane extract (400 g) gave 385 g of non-volatile residue which yielded 360 g ether soluble material. The latter was paritioned with C_6H_6–EtOH–H_2O (3:0.75:0.25) to give a C_6H_6 rich fraction, which after washing with ice cold 5% NaOH yielded 90 g of neutral material. Chromatography of 20 g of this neutral fraction on 1 kg of activity II Merck neutral alumina gave 6.3 g in the hexane eluent, 3.8 g in the 1:1 hexane–C_6H_6 eluent, 5.3 g in the 1:4 hexane–C_6H_6 eluent, 1.0 g in the C_6H_6 eluent, 1.7 g in the $CHCl_3$ eluent and 1.5 g in the MeOH strip.

From the early fractions of the 1:1 hexane–C_6H_6 eluent a white solid was deposited on evaporation of the solvent. Recrystallization from hexane gave friedelin as white needles, single peak by GLC on 180 × 0.6 cm 3% OV17 column. Mp 255–257 (uncorr.), mp 256–257° (266–267° *in vacuo*) [9]; ν_{max}^{KBr} 1710 cm^{-1}, ν_{max}^{KBr} 1709 cm^{-1} [9]; $[\alpha]_D$ −21 (*c*, 1.1 $CHCl_3$), $[\alpha]_D$ −19 to −29° [9]; M^+ *m/e* 426 (3%). Oxime mp 277–278; $[\alpha]_D$ +58° (*c*, 0.91, $CHCl_3$); mp 280–282° [9], $[\alpha]_D$ +56° [9]. Enol benzoate mp 257–258°, $[\alpha]_D$ +57° (*c*, 0.85, $CHCl_3$); mp 255–256° [9], $[\alpha]_D$ +59° [9].

Later eluates from the 1:1 hexane–C_6H_6 fractions deposited a solid (mp 275–277° from hexane, contaminated with friedelin) which was similar to friedelin in the gross feature of its IR and NMR spectra but showed strong O-H absorption in the IR In addition, this solid was indistinguishable from friedelin by GLC on a 5% SE-30 column and by TLC on Si gel. However, the solid was clearly distinguishable from friedelin on a 3% OV-17 GLC column and was shown to be friedelan-3α-ol as follows. Jones oxidation of the alcohol gave friedelin, identical with an authentic sample by GLC (3% OV17 column), IR, NMR and mp. The alcohol was converted into its benzoate by heating with benzyol chloride in pyridine. Mp 248–250° (1:1 $CHCl_3$–MeOH); mp 247–248° [10], the mother liquor yielded a second crop, mp 247–248°. Hydrolysis of the benzoate with 8% ethanolic KOH gave friendelan-3α-ol. Mp 302–303°; mp 300–301 [10]; $[\alpha]_D^{28}$ +17° (*c*, 1.38 $CHCl_3$), $[\alpha]_D$ +18° [11]; ν_{max}^{KBr} 3480, 3610 cm^{-1}; M^+ *m/e* 428 (5%), *m/e* 275 (15%) [12].

Acknowledgements—We express our sincere appreciation to the National Cancer Institute for support of this research (1-R01-

CA-18819-01). We also thank Mr. John Novak for preliminary separation and GLC.

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STEROLS AND FATTY ACIDS OF SOME NON-PHOTOSYNTHETIC ANGIOSPERMS*

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(*Received* 28 *March* 1977)

Key Word Index—*Cuscuta campestris*; Convolvulaceae; *Monotropa uniflora*; *M. hypopitys*; Pyrolaceae; fatty acids; sterols; non-photosynthetic angiosperms.

Abstract—Sterols and fatty acids were extracted and identified from three parasitic angiosperms, *Cuscuta campestris*, *Monotropa uniflora* and *M. hypopitys*. Each plant contained the typical 16 and 18-carbon fatty acids of angiosperms, but the partially-photosynthetic *Cuscuta* contained much larger quantities of linolenic acid than the non-green *Monotropa* species which had smaller amounts of linolenic acid characteristic of non-photosynthetic tissue. Sterol quantity was three times higher in *Cuscuta* than in the *Monotropa* species. Sitosterol was the major sterol in all species with smaller amounts of campesterol and cholesterol.

INTRODUCTION

While the isolation and identification of sterols and fatty acids in photosynthetic higher plants has been under extensive study for the past decade, research on the fatty acids and sterols of non-photosynthetic higher plants has been almost completely ignored. One report, by Rhomer *et al.* [1], determined that the sterols of two non-photosynthetic higher plants, *Cuscuta epithymum* and *Orobanche lutea*, were similar to the sterols of photosynthetic higher plants. The purpose of this study was to extract and identify the fatty acids and sterols of three non-photosynthetic seed plants common to eastern North America.

*Scientific Article No. A2277, Contribution No. 5274 of the Maryland Agricultural Experiment Station.

RESULTS AND DISCUSSION

The major fatty acid from *Cuscuta campestris* was linolenic acid, followed by linoleic acid and palmitic acid. Small amounts of stearic acid and oleic acid were also identified from the sample (Table 1).

Analyses of the sterols of *C. campestris* showed sitosterol as the major sterol, followed by campesterol and stigmasterol. A rather high concentration of cholesterol was identified in an amount similar to that of campesterol. In *C. epithymum*, cholesterol was found only in trace amounts [1].

Except for the presence of small amounts of hexadecenoic acid (16:1), the fatty acids of *Monotropa uniflora* were qualitatively similar to those of *Cuscuta*. However, the quantities of the individual fatty acids differed considerably. The major fatty acid for *M. uniflora* was linoleic acid, followed by palmitic acid.

Antifeedants from Rayless Goldenrod and Oil of Pennyroyal: Toxic Effects for the Fall Armyworm[1,2,3]

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Reprinted from the
JOURNAL OF ECONOMIC ENTOMOLOGY

Antifeedants from Rayless Goldenrod and Oil of Pennyroyal: Toxic Effects for the Fall Armyworm[1,2,3]

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ABSTRACT

J. Econ. Entomol. 72: 812–815 (1979)

The volatile oils and methanol-water partition fractions from rayless goldenrod, *Isocoma wrightii* (Gray) Rbd., at concentrations of 20,000 and 50,000 ppm, respectively, inhibited the feeding of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), almost completely, while, oil of pennyroyal, *Mentha pulegium* (Linn.), inhibited feeding completely at 5000 ppm. When these goldenrod fractions were incorporated in the insects' rearing diet at 1000 ppm concentration, the following toxic effects were noted: decrease in the weight of the larvae and number surviving on the 12th day; decrease in the number of normal adult moths emerging; and increase in the total days of the life cycle. At this concentration with the oil of pennroyal all of the larvae died within 24 h when placed on the medium.

Although "secondary plant metabolites" with their challenging structures and biosynthetic and metabolic pathways have intrigued scientists for many years, it is only recently that an understanding of the vital role these compounds play as a basis of insect host selection and as resistance factors in protecting the plant against insect attack has developed. These allelochemicals may serve as natural antifeedants, toxins, or may exert a chronic effect on the rate of growth and development of the insect. By detecting and isolating these compounds in the laboratory, natural environment chemicals which are specific and which will overcome the serious problems of synthetic insecticides can be found. In screening a variety of plant extracts for antifeedant activity using the leaf disk method (Wada and Munakata 1968) with the polyphagous fall armyworm, *Spodoptera frugiperda* (J. E. Smith) it was found that the ethanol extract from rayless goldenrod, *Isocoma wrightii* (Gray) Rbd., and the votatile oils from pennyroyal, *Mentha pulegium* (L.), had significant activity. The former plant, which is found west of the Mississippi River, is known to have other biological effects such as causing "trembles" in higher animals that is passed to humans through milk as "milksickness" (Furbee and Snively 1968, Christensen 1965), while the latter has been effectively used to repel ants and fleas (Griffith-Jones 1978).

In order to locate the specific antifeedant(s), the plant was extracted and partitioned as illustrated in Fig. 1 using the leaf disk assay (Wada and Munakata 1968) to locate the activity. Specific compounds isolated were also tested for antifeedant activity. Since allelochemicals can produce a chronic effect on insects, some of the partition fractions and compounds isolated were added to the artificial rearing diet to investigate this effect.

Materials and Methods

After drying, rayless goldenrod collected from New Mexico was extracted with 95% ethanol. This extract (F1) was then partitioned according to the procedure in Fig. 1. Purification of the compounds was accomplished by chromatography of the partition fractions, on silica gel eluting with mixtures of hexane-ethyl acetate of increasing polarity.

The oil of pennyroyal was obtained from Fritzsche Bros., New York, and was found to consist of 85% pulegone by GLC (15.2×0.32 cm, 5% SE 30, 160°). To assay for antifeedant activity, leaf disks (18 mm diam) were punched out of leaves from black-eyed pea plants, *Vigna unguiculata* (L.) Walp; these plants were at least 10 days old. When enough for a whole experiment had been prepared, 8 were randomly selected and dipped in acetone solutions of plant extract (Table 1) or compound for 5 sec, then were air dried before placing them in a covered petri dish (14 cm diam × 2 cm deep) which had filter paper moistened with water in it. The control consisted of 8 disks dipped in acetone. After starving for 2 h, five 6th-instar fall armyworms were placed in the dish and allowed to feed in the dark at 25°±1°C for 2 h. After removing the insects from the dish, the amount eaten was measured by the method of Dethier (1947) which involved measuring the area of the disks that had been eaten in each dish. The feeding ratio or the area of the test eaten compared to the control (test/control) was calculated. Each experiment was done in triplicate. The antifeedant activity is reported for each feeding ratio as follows: +4(0.00–0.10); +3(0.10–0.30); +2(0.30–0.50); and +1(> 0.50). These are listed in Table 1 as well as in the partitioning scheme, Fig. 1.

The following compounds from the volatile oils (F6) were tested at a concentration of 4000 ppm; β-caryophyllene, caryophyllene oxide, isocomene, (+) limonene, (−) carvone, (−) borneol, bornyl acetate and modhephene (Zalkow et al. 1977, 1978, 1979a)[4]. None of these showed antifeedant activity. The following compounds from F8 were assayed at 4000 ppm: the benzofurans, toxol, toxyl angelate and 2,5-diacetyl benzofuran and *cis*-7-[3-methyl-2-butenyl)oxy] coumarin (Zalkow et al. 1979b)[4]. Among these, only toxyl angelate had significant activity with a feeding ratio of 0.15.

To determine the antibiotic effect of the allelochemicals on the fall armyworm, plant extracts and pure compounds were incorporated in the insects' standard rearing diet (Shorey and Hale 1965). The extracts and

[1] Lepidoptera: Noctuidae.

[2] Received for publication May 23, 1979.

[3] Presented in part at The American Society of Pharmacognosy and the Phytochemical Society of North America Meeting, Oklahoma State University, Stillwater, Aug. 1978; American Chemical Society, Southeastern Regional Meeting, Savannah, GA, Nov. 1978; and American Chemical Society, Middle Atlantic Regional Meeting, Monmouth College, Long Branch, NJ, Mar. 1979.

[4] The isolation and identification of these compounds have been reported in the references cited.

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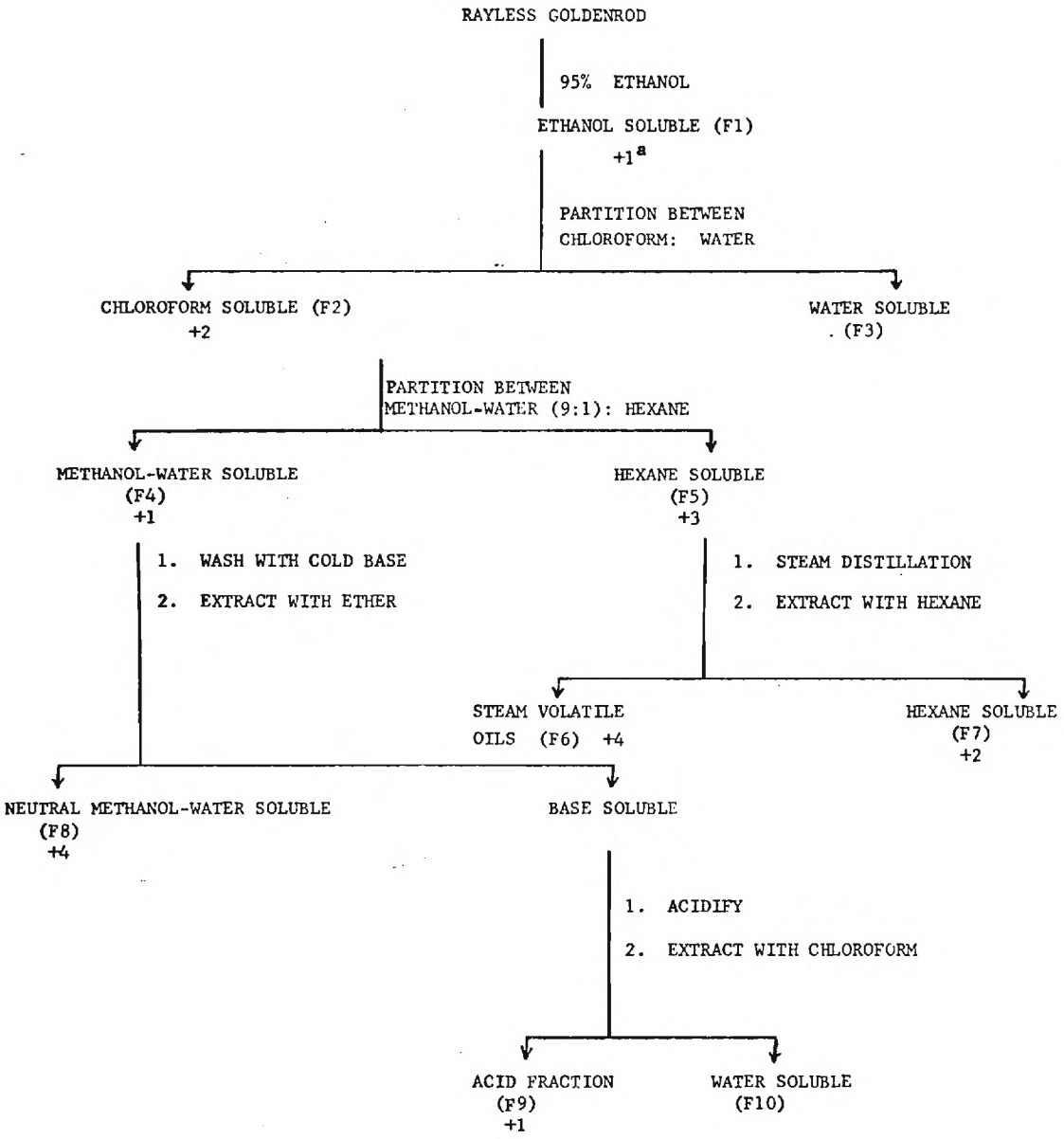

[a] See footnote a in Table 1.

FIG. 1.—Partitioning procedure for rayless goldenrod extract.

chemicals (Table 2) were weighed and dissolved in ethanol (2 ml) and added to the weighed hot medium during preparation. In the control, the same quantity of ethanol was also added. For each experimental group, 15 2nd instars were put separately in beakers which contained the diet and reared at 25°±1°C. The weight and development of the insects was measured at intervals.

Table 2 gives results from these experiments. To compare the development of the test larvae with those of the control, larvae were weighed on the 12th day after hatching, and the ratio of the weight of the larvae reared with additives in the diet to that of their respective controls was calculated. The ratio of surviving insects on the 12th day was also determined. Before ending the experiment, the ratio of normal adults from the larvae that had pupated was noted and calculated as was the

Table 1.—Antifeedant activity for the fall armyworm from rayless goldenrod and oil of pennyroyal fractions.

Fraction	Concn ppm	Feeding ratio test/control	Antifeedant activity[a]
Rayless Goldenrod			
Ethanol (F1)	50,000	0.65	+1
Chloroform (F2)	50,000	0.34	+2
Methanol-water	50,000	0.45	+1
(F4)	50,000	0.11	+3
Hexane (F5)	10,000	0.42	+1
Volatile oils (F6)	20,000	0.05	+4
	10,000	0.30	+3
	2,000	0.40	+2
	1,000	0.62	+1
Hexane after steam distillation (F7)	50,000	0.32	+2
Methanol-water neutral (F8)	50,000	0.05	+4
	20,000	0.15	+1
Methanol-water acid (F9)	50,000	0.45	+1
Oil of Pennyroyal			
Volatile oils	5,000	0.00	+4
	1,000	0.26	+3
	100	0.71	+1

[a] The extent of antifeedant activity was measured as follows: those with a feeding ratio between 0.00–0.10 were the most active and are denoted with an antifeedant activity of +4, +3 for a feeding ratio of 0.10–0.30, then +2 for 0.30–0.50 and +1 for > 0.50.

number of days from the hatching of the larvae to the emergence of adult moths for those that completed the whole cycle. The ratio of normal adults in the test to the control was calculated from those insects that emerged from the pupae alive and without visiable defects.

Results and Discussion

From the results of the leaf disk assay in Table 1, it is seen that the most active fraction is the volatile oil (F6) with 100% feeding inhibition at a concentration of 20,000 ppm. By using decreasing amounts of F6, it is seen that this activity decreases to a feeding ratio of 0.62 at 1000 ppm. The neutral methanol-water soluble fraction (F8) is also active with a feeding ratio of 0.05 at 50,000 ppm and 0.15 at 20,000 ppm; but only one compound isolated from either of these fractions (F6 or F8), the benzofuran toxyl angelate, was significantly active with a feeding ratio of 0.15 at 40,000 ppm. However, since oil of pennyroyal had complete inhibition of feeding at 5000 ppm and still showed activity (0.26) at 1000 ppm, it is the most potent antifeedant tested. This oil is composed mostly of the monoterpene pulegone (85%).

Table 2 demonstrates the detrimental effect of various components from rayless goldenrod and oil of pennyroyal when added to the rearing diet of the fall armyworm. In almost every case, there also was a decrease in the number of insects surviving on the 12th day, a decrease in the number of normal emerging adult moths, and an increase in the length of the life cycle from hatching to emergence. The volatile oil fraction (F6), which had the most antifeedant activity, showed the largest overall effect in this test with a weight ratio of 0.37. Only 75% survived on the 12th day and 77% normal adults emerged as compared to the control, while the life cycle was extended to an average of 33.0 days, 4.5 days longer than the control. Since toxyl angelate was the only compound isolated which showed any significant antifeedant activity, it and the related benzofurans toxol and 2,5-diacetyl benzofuran were included in the rearing experiment. Among these, as seen in Table 2, toxol exhibited the greatest overall toxic effect with a weight ratio of 0.58 and 0.73 survival ratio on the 12th day; the emergence ratio was 0.53 and the life cycle was extended to 34.2 days. However, when oil of pennyroyal, the most potent of the antifeedants, was added to the rearing diet at a concentration of 1000 ppm all of the larvae were killed within 24 h; at 100 ppm, 10 larvae were killed within 48 h, while the remaining 5 did not develop normally.

As can be seen from these results, a number of additives were antifeedants at high concentrations, but when lower concentrations were used the antifeedant effect was greatly reduced but overall toxic effect remained. Practically speaking, while an insect may choose to eat a plant containing a weak antifeedant rather than starving, this choice could ultimately result in a reduction of its population due to toxic effects. With development,

Table 2.—Toxicity of additives in rearing diet to the fall armyworm.

Additives	Concn additive (ppm)	Wt ratio test/control on 12th day[a]	Survival ratio test/control on 12th day	Ratio normal adults test/control	No. of days to adult
Control	0.000	1.00	1.00	1.0	24.5
Rayless Goldenrod					
Ethanol extract (F1)	5,000	0.52	0.93	0.77	28.0
	1,000	0.58	0.80	0.54	25.6
Methanol-water (F8)	1,000	0.55	1.00	0.92	32.7
Methanol-water (F4)	1,000	0.73	0.66	0.77	33.6
Volatile oils (F6)	1,000	0.37	0.75	0.77	33.0
Toxol	500	0.58	0.73	0.53	34.2
Toxyl angelate	500	0.48	0.73	0.69	33.0
2,5-diacetylbenzofuran	500	0.32	0.93	1.00	32.6
Oil of Pennyroyal					
	1,000[b]	—	0.00	—	—
	100[c]	0.29	0.34	—	—

[a] The difference between the test and control were found to be significant at the 0.05% level by the "*t*-test".
[b] Second and third instars died within 24 h after being placed on medium.
[c] Ten larvae were dead within 48 h, while the remaining 5 did not develop normally.

antifeedants may provide another method of controlling insects in the field or during crop storage.

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